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Screening techniques and sources of resistance to foliar diseases caused by major necrotrophic fungi in grain legumes

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Summary

Necrotrophic pathogens of the cool season food legumes (pea, lentil, chickpea, faba bean and lupin) cause wide spread disease and severe crop losses throughout the world. Environmental conditions play an important role in the development and spread of these diseases. Form of inoculum, inoculum concentration and physiological plant growth stage all affect the degree of infection and the amount of crop loss. Measures to control these diseases have relied on identification of resistant germplasm and development of resistant varieties through screening in the field and in controlled environments. Procedures for screening and scoring germplasm and breeding lines for resistance have lacked uniformity among the various programs worldwide. However, this review highlights the most consistent screening and scoring procedures that are simple to use and provide reliable results. Sources of resistance to the major necrotrophic fungi are summarized for each of the cool season food legumes. Marker-assisted selection is underway for Ascochyta blight of pea, lentil and chickpea, and Phomopsis blight of lupin. Other measures such as fungicidal control and cultural control are also reviewed. The emerging genomic information on the model legume, *Medicago truncatula*, which has various degrees of genetic synteny with the cool season food legumes, has promise for identification of closely linked markers for resistance genes and possibly for eventual map-based cloning of resistance genes. Durable resistance to the necrotrophic pathogens is a common goal of cool season food legume breeders.

Introduction

The cool season food legumes (faba bean, chickpea, pea, lentil and lupin) play an important role in the farming systems worldwide (Kelley et al., 1997). Protein concentrations range from 22% in pea to 45% in lupin. They are used for human food and animal feed throughout the world. These crops are attacked

by aerial fungal pathogens that cause diseases such as grey mould, chocolate spot, ascochyta blight, anthracnose, rust, powdery mildew and downy mildew. These diseases are caused by fungi classified either as necrotrophic or biotrophic. The former are defined by Agrios (2004) as organisms that infect, colonize and kill living tissue to obtain energy to grow and multiply. The latter are defined as fungi that grow and reproduce in nature only in living hosts and are referred to as obligate parasites.

The relative importance of aerial fungal diseases and their effect on yield varies among years and cropping regions. However, some of them affect large areas in all the countries where legumes are cultivated and cause considerable losses in quality and quantity. The major necrotrophic fungal diseases are ascochyta blight on various grain legumes, chocolate spot on faba bean and anthracnose of lupin and lentil.

Control of these diseases is based on cultural management, the use of chemicals, genetic resistance or some combination of these approaches. Disease resistance is currently a primary objective of most plant breeding programs. Durable and multi-disease resistance is considered as a prerequisite to broad environmental adaptation aiming at stabilising agricultural systems (Cowling, 1996).

Fungal disease development on host plants is a step-by-step phenomenon, starting with a contamination phase, corresponding to contact between propagules (usually spores) of the fungus and the host plant. In the event of adequate receptivity and compatibility, spores germinate and can form fixation structures (apressoria) that allow the fungus to penetrate the host either directly through the cuticle, through stomates or through wounded tissues. Penetration is followed by an infection phase, where the fungus will settle and invade the host tissues, ensuring its development either on living tissues (biotrophic fungus) or on dead tissues (necrotrophic fungus). This phase results in the symptom development. The fungus then develops specialised structures aiming at sporulation and leading to the production of secondary inoculum that will be disseminated and contribute to the start of another infection cycle. In the case of pathogens having several cycles per growing season, the number of secondary infection cycles usually determines the intensity of epidemics in the field.

Complete resistance interferes with the disease cycle by totally preventing the emergence of symptoms and/or the production of spores, thereby preventing pathogen multiplication. This resistance is usually based on a qualitative response. Partial resistance interferes with one or more of the steps of the cycle, resulting in slowing disease progress and/or reducing pathogen multiplication (Parlevliet, 1979). Partial resistance must be assessed on a quantitative scale, while relying on certain aspects of disease epidemiology. For example, the growth of the pathogen can be assessed quantitatively by direct evaluation of disease severity (symptoms) and disease development over time, or by considering disease severity as the result of several factors (Parlevliet, 1979) or components of partial resistance such as:

- resistance to infection, i.e. reduced spore germination, apressoria formation or penetration;
- delayed incubation period, covering the time between inoculation and the appearance of the first symptoms;
- delayed latency period, covering the time between inoculation and sporulation;
- reduced infectious period, e.g. the duration of sporulation;
- reduced spore production.

The interaction between the pathogen and the host defines race specificity or non-race specificity of resistance and is based on the presence or absence of statistically significant interaction between host and pathogen genotypes. Resistance is said to be specific when the reactions of a set of hosts (called differentials) differ when confronted by different isolates of the pathogen. These specific strains are referred to as races. Pathogenicity of the pathogen comprises virulence and aggressiveness which refer respectively to the qualitative capacity of a strain to infect a specific host genotype and to the quantitative capacity of a strain to infect a set of host genotypes (Rapilly, 1991). Aggressiveness can be an important factor in disease development and is defined as the severity of disease and its ability to invade host tissue over a given time period (Ribeiro do Vale et al., 2001). Pathotype can be defined as an intraspecific group of isolates characterized by similar levels of virulence (or pathogenicity) on a given set of host genotypes.

Evaluation of resistance depends on several crucial factors including the choice of appropriate isolates for screening, screening methodology (under controlled conditions or field conditions) and the sources of resistance. Our objective is to review screening techniques and sources of resistance of grain legumes to foliar diseases caused by major worldwide necrotrophic fungi.

Necrotrophic foliar diseases of lentils

Foliar diseases including ascochyta blight, anthracnose, and grey mould are considered the most serious biotic constraints to lentil production (Bayaa & Erskine, 1998).

Ascochyta blight

Ascochyta blight, caused by *Ascochyta lentis* Bondartsev and Vassilievskii (syn. *A. fabae* Speg. f. sp. *lentis*), has been reported worldwide in most lentil-producing countries (Bayaa & Erskine, 1998). The disease is characterised by necrotic lesions on leaves, stems and pods that may lead to significant yield loss, seed discolouration and reduced seed quality (Gossen & Morrall, 1983). While the use of fungicides, disease-free seed and crop rotation have been recommended for disease control, the most economical and efficient method for controlling ascochyta blight in lentils is through the use of resistant germplasm.

Choice of appropriate isolates

Pathogenic variability has been reported in populations of *A. lentis*. Nasir and Bretag (1997b) found that 39 isolates of *A. lentis* could be divided into six distinct pathotypes based on the reactions of ILL358, ILL7537, ILL7515, ILL5588, ILL5244 and Eston to infection. A significant finding from this work was a single isolate that was virulent to ILL5588. To date, ILL5588 has maintained resistance under field conditions in Australia. Kaiser et al. (1994) also reported that isolates of *A. lentis* collected off seed from 17 countries varied in growth, sporulation, colony appearance and morphology but all were pathogenic on two susceptible lentil cultivars in Canada. Ahmed and Morrall (1995) found an increase in virulence when comparing 1978–1979 isolates of *A. lentis* to isolates collected in 1991. This was demonstrated by the apparent change in susceptibility of the cultivar Laird, which was rated as the fifth most susceptible of the 10 differential cultivars to the 1978–1979 isolates, but became the most susceptible cultivar when exposed to the 1991 *A. lentis* isolates. However, in the analysis of the reactions of the 10 differentials to the isolates, the contribution of differential $\times$ isolate interaction to variability in virulence was very low and did not suggest the existence of distinct races in *A. fabae* f. sp. *lentis*.

Techniques for screening

Field screening. Resistance screening in the field has been commonly used to identify lentil germplasm resistant to ascochyta blight. Often "disease nurseries" are sown in areas where natural disease pressure is high; this may be in districts where environmental conditions are conducive for disease or where a large source of inoculum is present, for example a field previously sown to lentil. This allows test entries in the nursery to be exposed to the prevailing pathogen population and hopefully account for variability within the *A. lentis* population. Disease pressure can be further increased by the use of disease spreader rows throughout the field trial. These are naturally highly susceptible genotypes, which act as inoculum sources or "foci" of infection, and add disease pressure onto adjacent test entries. These may be arranged either as designated blocks throughout the evaluation trial, to ensure an even spread of disease, or as a running check after a given number of test entries. Often diseased host stubble may be spread over the test site to initiate disease epidemics across the trial site, or artificial inoculum may be applied in the form of a spore suspension. Screening for resistance to ascochyta blight in the field can sometimes be difficult due to the indeterminate growth habit of lentil and the tendency of lentil to shed diseased leaflets (Chowdhury et al., 2001), often during periods of stress. This often requires several disease ratings or measures during the growing season to account for the loss of diseased foliage (Chowdhury et al., 2001; T. Bretag, personal communication). In Australia, seed harvested from evaluation trials is also tested for levels of seed infection and discolouration. Under Australian conditions, screening germplasm in the field has been highly successful in identifying germplasm resistant to foliar and seed infection by *A. lentis*.

Controlled conditions. Pathogenic variability within *A. lentis* requires caution to be taken if screening activities are to be based on the use of a single or few *A. lentis* isolates. The use of check cultivars to ensure differentiation between susceptible and resistant cultivars can alleviate concerns with the use of a single isolate. The screening of germplasm under glasshouse conditions often allows cultivars to be screened rapidly, using techniques that are easily reproducible and in some instances during periods when environmental conditions in the field are not conducive for disease development. Under controlled conditions, lentil germplasm can be subject to pathogenic isolates at the researcher's discretion. Nguyen et al. (2001) used a selected number

of *A. lentis* isolates of varying pathogenicity to identify genes for resistance in the cultivar ILL7537.

Studies under controlled conditions allow epidemiological factors to be observed in detail that may otherwise be affected by other biotic or abiotic stresses under field conditions. Pedersen and Morrall (1994a,b) investigated the effects of cultivar resistance, duration of leaf wetness following inoculation, temperature and growth stage on infection and development of *A. lentis* under controlled conditions. Resistant genotypes were found to have less lesions and pycnidia production than more susceptible genotypes.

To incite disease on lentil seedlings researchers have used various spore concentrations of *A. lentis*, varying from 5×10^4 (Singh et al., 1982), 7.5×10^4 (Ye et al., 2003), 1×10^5 (Nasir & Bretag, 1998), to 5×10^5 (Tar'an et al., 2003a,b). Dew periods following inoculation are commonly 48 h duration.

Sources of resistance

Genetic variability for resistance to foliar ascochyta blight in lentil has been reported from Argentina (Khare et al., 1993; Erskine et al., 1994), Syria (Erskine & Saxena, 1993; Khare et al., 1993; Erskine et al., 1994), Pakistan (Iqbal et al., 1990; Hussain et al., 2000), India (Singh et al., 1982; Kapoor et al., 1990; Sugha et al., 1991), Ethiopia (Ahmed & Beniwal, 1991) and Australia (Nasir & Bretag, 1998). A high level of foliar resistance has also been identified in the wild *Lens* species *culinaris* ssp. *orientalis*, *L. odemensis*, *L. nigricans*, *L. ervoides* and *V. montbretii* (Bayaa et al., 1994; Ahmad et al., 1996; Ye et al., 2000). In Canada, PI 339283, ILL5588, PR86-360 and PI 374118 have shown high levels of resistance to foliar and seed infection by *A. lentis*. In the same experiment, Laird was moderately resistant to foliar infection but susceptible to seed infection and Indianhead was highly resistant to foliar infection and moderately resistant to seed infection (Andrahennadi et al., 1996). In Australia, Nasir and Bretag (1997b) found ILL358 and ILL7537 to be resistant to foliar infection after screening with 39 different isolates of *A. lentis*; in addition, seed infection by *A. lentis* has been found to be lowest in the cultivars Northfield (ILL5588), ILL7193 and ILL7199 (Nasir & Bretag, 1997a).

Cultivars reported with resistance to *A. lentis* include Manserha 89 in Pakistan (Erskine & Saxena, 1993; Erskine et al., 1994), Pant L4 (Singh et al., 1994) and Masoor 93 (Tufail et al., 1995) in India, Rajah (ILL 6343) in New Zealand (Russell, 1994) and CDC Milestone, CDC Glamis, CDC Grandora, CDC Sovereign, CDC Vantage and CDC Robin in Canada (Vandenberg et al., 2001, 2002a,b,c,d,e). In Australia Northfield (ILL5588) is the only current commercial cultivar with resistance to both foliar and seed infection by *A. lentis* (Ali, 1995).

Genes conferring foliar and seed resistance to *A. lentis* have been identified in cultivated and wild lentil species, these are summarised in Tables 1 and 2. Comparisons between studies are difficult due to the differences in screening methodologies and scoring systems used. From the summary tables it is clear that foliar resistance has been the focus of many genetic studies with lentil. Ye et al. (2003) indicate that the relationship between foliar resistance and seed resistance is not entirely clear. This becomes further complicated when many researchers have used resistance to seed infection and subsequent seed infection rates as a measure of cultivar resistance and not foliar resistance alone.

Anthracnose

Colletotrichum truncatum (Schwein.) Andrus and W.D. Moore is a common and important pathogen on lentil in Canada (Morrall, 1988; Anderson et al., 2000a), and is listed as a minor disease in several countries in Asia and Africa by Bayaa and Erskine (1998). Strictly speaking, the fungus is a hemibiotroph with a short biotrophic phase of 48 h prior to switching to necrotrophic colonization of the host (O'Connell et al., 1993; Chongo et al., 2002). *Colletotrichum truncatum* causes anthracnose characterized by necrotic lesions with depressed centres which are initially concentrated on the stem base and lower branches before spreading to leaves and pods (Bernier et al., 1992). Wilting, extensive defoliation, poor pod fill and plant death are common symptoms in severely infected lentil fields (Gibson, 1994). The fungus spreads *via* conidia during the season, and through microsclerotia, infected seed and infected lentil stubble between seasons (Buchwaldt et al., 1996). The epidemiology of the fungus on lentils has been intensively studied in Canada (Anderson et al., 2000b; Chongo & Bernier, 2000a,b).

Choice of appropriate isolates

C. truncatum has been isolated from a wide range of plant species, primarily belonging to the *Leguminosae* (Sutton, 1998). Isolates from lentil are highly pathogenic on faba bean (*Vicia faba* L.), grasspea (*Lathyrus sativus* L.) and vetch (*Vicia* spp.), but cause only weak symptoms on chickpea (*Cicer arietinum* L.), bean (*Phaseolus vulgaris* L.), soybean (*Glycine max*

Table 1. Inheritance of foliar resistance to *Ascochyta lentis* in cultivated and wild lentil species

Resistant genotype	Susceptible genotype (*L. culinaris*)	Gene control	References
ILL5588 (*L. culinaris*)	Eston	One dominant gene	Tay and Slinkard (1989)
ILL5588 (*L. culinaris*)	ILL6002	One dominant gene	Ford et al. (1999)
ILL5588 (*L. culinaris*)	Titore	Two dominant genes	Ye et al. (2001a, 2003)
ILL5684 (*L. culinaris*)	Eston	One dominant gene	Tay and Slinkard (1989)
ILL5684 (*L. culinaris*)	Titore	One dominant gene	Ye et al (2001b, 2003)
ILL7537 (*L. culinaris*)	IC128/85	Two dominant complementary genes	Nguyen et al. (2001)
Indianhead (L. culinaris)	Titore	Two additive recesssive genes	Ye et al. (2001a, 2003)
Indianhead (*L. culinaris*)	Eston	One recessive gene	Chowdhury et al. (2001)
Laird (*L. culinaris*)	Eston	One recessive gene	Tay and Slinkard (1989)
Laird (*L. culinaris*)	Titore	One recessive gene	Ye et al. (2001a, 2003)
W6 3241 (*L. orientalis*)	Invincible	One dominant gene	Ahmad et al. (1996)
W6 3241 (*L. orientalis*)	Titore	One dominant gene	Ye et al. (2001b)
W6 3261 (*L. orientalis*)	Titore	One dominant gene	Ahmad et al. (1996)
W6 3261 (*L. orientalis*)	Olympic	One dominant gene	Ahmad et al. (1996)
W6 3261 (*L. orientalis*)	Invincible	One dominant gene	Ahmad et al. (1996)
W6 3261 (*L. orientalis*)	Invincible	Two dominant genes	Ye et al. (2001a)
W6 3192 (*L. ervoides*)	Titore	Two dominant complementary genes	Ahmad et al. (1996), Ye et al. (2003)
W6 3192 (*L. ervoides*)	Olympic	Two dominant complementary genes	Ahmad et al. (1996)
W6 3222 (*L. odemensis*)	Titore	Two dominant complementary genes	Ahmad et al. (1996)

Summary table compiled from Materne et al. (2002) and Ye et al. (2002).

Table 2. Inheritance of seed resistance to *Ascochyta lentis* in cultivated lentil

Resistant genotype	Susceptible genotype (*L. culinaris*)	Gene control	References
Indianhead	PI 345635	Two duplicated recessive genes	Andrahennadi (1994)
Laird	Eston	One recessive gene	Tay and Slinkard (1989)
ILL5588	Eston	One dominant gene	Andrahennadi (1994)
ILL5588	Eston	Two dominant genes, one recessive gene	Tay (1989)
ILL5588	Eston	One dominant gene, one recessive gene	Sakr (1994)
ILL5684	Eston	One dominant gene	Tay and Slinkard (1989)
ILL5684	Eston	Two dominant genes	Tay (1989)

Summary table compiled from Materne et al. (2002) and Ye et al. (2002).

(L.) Merr.) and alfalfa (*Medicago sativa* L.) (Gibson, 1994; Anderson, 2003). Morphological examination of conidia produced by isolates from different hosts revealed that *C. truncatum* from different host plants had similar dimensions. However, whereas those from lentil were ellipsoidal, isolates from *Glycine max*, *Xanthium occidentale* and *Arachis hypogaea* were falcate (Ford et al., in press). Using RAPD markers and 18-25S rDNA data, it was also possible to differentiate between isolates from different hosts, and conserved nuclear DNA was used to develop a highly specific molecular marker to identify lentil isolates in pure culture as well as in infected plant material (Ford et al., in press).

Detailed population studies on lentil isolates were conducted in Canada to identify pathogenic variation using 'Eston' as a susceptible check, and seven resistant accessions (PI 320937, PI 345629, PI 468901, Lens 102, Lens 104, Lens 195, Indianhead) identified through large-scale screening of germplasm (Anderson, 2003; Buchwaldt et al., 2003). Among 50 isolates collected from across the lentil growing area

in Canada, two distinct pathogenic groups were identified. One group caused severe symptoms on the susceptible variety Eston but was considered avirulent on the seven resistant differentials, and was designated Ct1. The second group was highly virulent on all varieties and accessions tested, and was designated Ct0. The ratio of Ct0 to Ct1 in the sample was 60:40. Mating of isolates of the two races has been initiated with the objective to elucidate the genetics of virulence, and sexual structures have been observed (C. Cho & S. Banniza, unpublished data). Representatives of both races are now used routinely to screen breeding material for resistance to anthracnose.

Techniques for screening

Field screening. Screening of lentil germplasm has been conducted under field conditions as well as greenhouse conditions (Buchwaldt et al., 2003; Tar'an et al., 2003a,b; Tullu et al., 2003, 2005). In both approaches, artificial inoculation was employed. Inoculation under field conditions has either relied on spore suspensions (Buchwaldt et al., 2003), infected residue from previous trials (Tullu et al., 2005), or sterilized wheat grains colonized with the pathogen prior to inoculation (Tullu et al., 2005). For inoculation with a spore suspension, plots were saturated with water prior to inoculation to increase relative humidity when plants reached the late vegetative or early flowering stage (44–49 days after seeding). Plants were spray-inoculated with a spore suspension of a single isolate at a concentration of 4×10^4 spores ml^{-1} applied at a rate of approximately 600 ml of suspension per meter square. Inoculation took place in the late afternoon to avoid high temperatures and polyethylene sheets on an A-frame were used to cover the plots during the night to maintain high relative humidity (Buchwaldt et al., 2003). Chopped up infected lentil straw from disease nurseries of the previous year or colonized wheat grains were spread in experimental plots by Tullu et al. (2005). Sterilized wheat grains were inoculated with an isolate of Ct1 and Ct0, respectively, incubated for 10 days, dried and repeatedly spread in the plots at a rate of 10 g m^{-2} ever 2 weeks until good infection levels were obtained (Tullu et al., 2005). Routine disease screening in the Canadian lentil breeding program relies on field screening to eliminate susceptible material using a disease nursery that has been enriched with natural inoculum over many years of continuous testing. Further screening of resistant material from the field nursery is conducted under controlled conditions.

Controlled conditions. For greenhouse experiments, accessions are planted in 9 or 10 cm pots together with a susceptible and resistant control plant at a density of six to eight seeds per pot, using three pots as replicates (Buchwaldt et al., 2003; Tar'an et al., 2003a,b; Tullu et al., 2003, 2005). Plants are inoculated with single isolate suspensions of 10^5 conidia ml^{-1} and 1.5 ml per plant 3–4 weeks after planting (10–12-node stage or early flowering). To avoid cross-contamination in experiments where different isolates are used (Buchwaldt et al., 2003), or where humidity around the plants has to be increased (Tullu et al., 2003), polyethylene wrapping is used. Plants are incubated for 24 h at 100% humidity, and then maintained in the greenhouse with 12-h supplemental light. Diseases symptoms are rated when the susceptible control plants start wilting (10–14 days after inoculation) (Tar'an et al., 2003a,b; Tullu et al., 2003, 2005) or 15–18 days (Buchwaldt et al., 2003) after inoculation using a semi-quantitative rating scale with five classes based on Buchwaldt et al. (2003): highly resistant (HR): no lesions on the stem; resistant (R): 1–30 lesions, only superficial, may occur on the whole stem; moderately resistant (MR): 1–10 lesions, either a few deep lesions on the stem base or a mixture of superficial and deep lesions on the lower half of the stem; moderately susceptible (MS): 15–20 lesions, a mixture of superficial and deep lesions including the top half of the stem, no shoot die-back or wilting; susceptible (S): 25–30 lesions, deep lesions including the top half of the stem, accompanied by shoot die-back or partial wilting; highly susceptible (HS): >30 lesions, numerous coalescent stem lesions, the plant half or completely wilted.

Sources of resistance

After discovery of the disease in Canada and severe outbreaks in the late 1990s, commonly grown lentil varieties in North America were screened for resistance to anthracnose and were found to be highly susceptible (Gibson, 1994). Extensive screening of more than 1500 accessions from the world collection of lentil from the U.S. Department of Agriculture – Agriculture Research Service (USDA-ARS, Pullman, WA, USA), from the International Centre for Agricultural Research in the Dry Areas (ICARDA, Aleppo, Syria), and from the Institute for Plant Genetics and Plant Research (Institut für Pflanzengenetik und Kulturpflanzenforschung, IPK, Gatersleben, Germany) under field and controlled conditions failed to reveal genotypes with complete resistance to the pathogen (Bernier et al., 1992; Buchwaldt et al., 2003). However,

16 accessions, including cultivars Indianhead (PI 320952, originating from the former Czechoslovakia), PI 468901 (originating from Brazil), PI 345629 (originating from the former USSR), and PI 320937 (originating from Germany), were identified with high levels of resistance characterized by leaf lesions, but a reduced rate of defoliation, superficial stem lesions, and reduced seed infection (Bernier et al., 1992; Buchwaldt et al., 2003). Crosses were made to introduce the resistance genes into lentil varieties with desirable agronomical traits (Buchwaldt et al., 1995). Genetic studies suggested that a single recessive gene conferred resistance in Indianhead, while that in PI 345629 and PI 320937 was based on single dominant resistance genes (Buchwaldt et al., 2001). The putative genes were designated *lct-1* (Indianhead), *LCT-2* (PI 320937) and *LCT-3* (PI 345629). Accessions with high levels of partial resistance were shown to have longer incubation and latent periods, developed smaller and fewer lesions, and had a lower percentage of sporulating stem lesions compared to the susceptible variety Eston (Chongo & Bernier, 1999). Microscopy studies of the infection process on PI 320937 and Eston showed that differences in the infection process occurred 48-h post-inoculation (Chongo et al., 2002). At that time, hyphal growth in PI 320937 was restricted, evident in the formation of aggregations, while the fungus proliferated in the stem and leaf tissue of Eston (Chongo et al., 2002).

When two races were identified in the population of *C. truncatum* (Buchwaldt et al., 2003; see 'Sources of appropriate isolate'), it was discovered that resistant accessions identified in screening tests, as well as the three Canadian lentil varieties (CDC Robin, CDC Redberry and CDC Viceroy) with anthracnose resistance released to date, had high levels of resistance to one race (Ct1), but were susceptible to the second (Ct0) (Buchwaldt et al., 2003). Since no source of resistance to Ct0 was found in *Lens culinaris*, other *Lens* species were screened including *L. orientalis* L. and *L. odemensis* L., which, together with *L. culinaris* L., are categorized as the primary gene pool, and *L. nigricans* (M. Bieb), *L. ervoides* (Brign.) Grande, *L. lamottei* Czefr. and *L. tomentosus* L., which are considered to be the secondary gene pool (Tullu et al., 2005). Accessions of these species were obtained from ICARDA, USDA-ARS and the Weizmann Institute at Rehovot in Israel, and screened under field and greenhouse conditions using isolates of Ct1 and Ct0 for artificial inoculation. Several accessions of *L. ervoides* had the highest level of resistance, some of *L. lamottei* and some of *L. nigricans* also showed medium levels of resistance, while accessions of the remaining species were susceptible (Tullu et al., 2005). Thus, the primary source of resistance to Ct0 was found in the secondary gene pool, introgression of which into *L. culinaris* is underway, using embryo rescue and micro-grafting techniques (A. Vandenberg, personal communication).

Botrytis grey mould

Botrytis grey mould (BGM) of lentils has worldwide distribution (Bayaa & Erskine, 1998), and has been noted as particularly damaging in Australia (Lindbeck et al., 2003), Bangladesh (Bahl et al., 1993), Canada (Morrall, 1997), Nepal (Karki, 1993), New Zealand (McKenzie et al., 1986) and Pakistan (Iqbal et al., 1992). In Asia and America only *Botrytis cinerea* has been implicated with BGM on lentils (Bayaa & Erskine, 1998). However, Davidson et al. (2004) have shown that both *B. fabae* and *B. cinerea* infect lentils and both may cause BGM in Australia, though their relative importance in the field is yet to be established. In Canada, *B. fabae* is considered to be the primary causal agent of this disease (Kucharan & Banniza, unpublished data). In established crops, under favourable conditions, infection develops on lower leaves, and then on stems. Lesions on stems are light brown or blanched and covered with grey mould and rot at the crown. Whole plants eventually become infected and dry out, to produce patches of dead plants. Pods and seeds may also become infected, producing discoloured, shrivelled seeds (Bayaa & Erskine, 1998).

Resistance to BGM in lentils is considered to be an essential part of an integrated disease management program, also incorporating fungicides and agronomic practices.

Choice of appropriate isolates

Minimal research has been conducted on the use of specific isolates for resistance screening to BGM of lentils. Kucharan et al. (2003) spray inoculated a field trial with a conidial suspension of 500 spores ml^{-1} using a mixture of randomly chosen isolates. Other screening trials have allowed BGM to develop through natural spread from *in situ* infected stubble (Materne et al., 2002; Davidson et al., 2004). In controlled conditions, it may be important that resistance screening requires the presence of both *B. cinerea* and *B. fabae* pathogens.

Techniques for screening

Field screening. To date, all resistance screening for BGM of lentils has been conducted in field nurseries. These have been inoculated with spore suspensions (Kucharan et al., 2003) or through natural spread from *in situ* infected stubble (Materne et al., 2002; Davidson et al., 2004). Disease is generally scored at flowering using a 1–9 scale, where 1: no disease and 9: dead plots, while Kucharan et al. (2003) scored five times through the season using the Horsfall–Barratt scale (0–11) scale. Variation among lines for BGM infection can be affected by several agronomic practices that produce a dense canopy and hence encourage disease epidemics (Bretag & Materne, 1998). These include early sowing dates and high seeding rates, row spacing, weed control and optimum fertiliser use, particularly high nitrogen levels (Bayaa & Erskine, 1998; Lindbeck et al., 2002). Stem strength, plant maturity and the level of leaf senescence may also affect disease levels. For example, observations in the field have indicated that genotypes with lodging resistance develop less disease as lodged plants create an ideal BGM microclimate. The development of more resistant lentil lines, in combination with current methods of disease management including agronomic practices and strategic use of fungicides, will increase the reliability and levels of production of lentil.

Controlled conditions. High humidity and temperatures of at least 20 °C are optimal if greenhouse screening of BGM on lentils is to be successful. Concentration of the *Botrytis* inoculum needs to be higher than 10^4 spores ml^{-1} to differentiate between resistant and susceptible lines in glasshouse conditions (J. Davidson, unpublished data). Senescent leaves and branches, and mature pods, can act as foci for BGM development on susceptible and resistant lines. In the greenhouse, disease development and severity is greatly exaggerated by the presence of senescent material, with a mere 2 days from inoculation to sporulation on the plants. The breakdown of resistance in the presence of senescent or mature material indicates that while resistant lines are coming through the breeding program, fungicides are likely to remain a part of controlling BGM in lentils (Davidson et al., 2004).

Sources of resistance

Resistance to BGM in lentils is poorly understood but must be determined to enable pyramiding of resistance genes. Screening for BGM resistance has just recently begun in Canada and Australia. BGM-resistant germplasm has been reported in Canada, where CDC Redcap, CDC Robin and CDC Milestone had consistently lower levels of disease in a field inoculated trial (Kucharan et al., 2003); in Nepal where genotypes Aarial, ILL2580, LG 171, LG 198, LN 0038 and Simrik were found to be resistant (Karki, 1993); in Pakistan where accessions ILL6004, ILL6016, ILL6024 were selected (Erskine et al., 1994; Tufail et al., 1993) and in Australia where the Canadian varieties Indianhead (ILL418) and Matador consistently rated resistant in Australian conditions (Materne et al., 2002).

Necrotrophic foliar diseases of lupin

The major foliar necrotrophic pathogens on lupin (*Lupinus angustifolius*, *L. luteus* and *L. albus*) are *Pleiochaeta setosa* Hughes which causes brown spot, *Diaporthe toxica* Will., Highet, Gams & Sivsith. (Phomopsis stem and pod blight) and *Colletotrichum lupini* (Bondar) Nirenberg, Feiler & Hagedorn which causes anthracnose. The occurrence, epidemiology and impact of these diseases were reviewed by Sweetingham et al. (1998). Of these, anthracnose is the most significant and is the focus of resistance breeding in major programs around the world. Anthracnose is often considered to be a hemibiotroph, but it clearly has a necrotrophic phase. Phomopsis is notable as the pathogen produces a potent mycotoxin that can kill livestock grazing the infected stubbles.

Anthracnose

Infected seed initiates hypocotyl and cotyledon lesions and conidia are rain-splashed to spread the disease. *C. lupini* infects all foliar parts but young stem, flower and pod tissue are the most susceptible. Both circumstantial and molecular evidence points to the existence of two closely related vegetative compatibility groups (Yang & Sweetingham, 1998; Nirenberg et al., 2002; Talhinhas et al., 2002) which appear to have spread globally with movement of seed (Gondran et al., 1994; Sweetingham et al., 1998). Both groups appear to have similar pathogenicity and host preference.

Techniques for screening

Several authors have reported glasshouse inoculation procedures that involve spray inoculation

of seedlings with a conidial suspension (typically 10^5–10^6 conidia ml^{-1}) and incubation under defined conditions of temperature and leaf wetness (Yang & Sweetingham, 1998; Wiatr et al., 2003). Suitable temperatures are in the range of 15–20 °C with post-inoculation leaf wetness periods of 12–36 h. Using such methods, Wells and Forbes (1967), Talhinhas et al. (2002), Wiatr et al. (2003) and Yang et al. (2004) were able to distinguish between resistant and susceptible varieties and breeding lines. To assess disease reaction, lesion severity is usually estimated on a scale relating to the size and extent to which lesions girdle the stem. As well as inoculum, temperature and moisture conditions, the growth stage of the seedlings was critical to ensure a uniform response. At the cotyledon stage all narrow-leafed lupins are very susceptible (G.J. Thomas, unpublished data).

However, with such artificial inoculations, variability in disease expression occurs from plant to plant even where great care is taken with all procedures. Thus, sufficient replication must be used to distinguish levels of resistance between genotypes. Yang et al. (2004) further modified the technique to enhance the ability to distinguish resistant and susceptible phenotypes in individual F2 plants. They got best results by inoculating the flower spikes after the flowers were carefully excised.

Field disease nurseries can be successfully established, particularly in high-rainfall environments or where overhead irrigation is available to supplement natural rainfall. Such nurseries typically require a layout whereby test plots are flanked by infected spreader rows of a susceptible genotype to ensure uniform disease pressure. Thomas and Sweetingham (2004) transplanted glasshouse-infected seedlings at regular intervals to initiate uniform infection. Under Australian conditions, early sowing increases epidemic development. Assessing disease severity in field nurseries can involve individual plant or whole plot rating scales depending on the level of precision required and resources available.

To provide more quantitative data on the relative resistance of cultivars, yield-loss experiments have been undertaken. Wiatr et al. (2003) correlated anthracnose susceptibility in *L. luteus* cultivars to relative grain yield with and without fungicide protection. Thomas and Sweetingham (2004) compared the yield of resistant and susceptible *L. angustifolius* cultivars under different disease pressures created by different levels of initial seed infection.

Sources of resistance

At a species level *L. albus* is most susceptible, followed by *L. luteus* and *L. mutabilis* and *L. angustifolius* is the most resistant (Cowling et al., 1999).

Weimer (1952) reported the first sources of resistance in *L. angustifolius* and *L. luteus* from wild and landrace accessions from Spain and Portugal from the USDA Plant Introduction collection. This source of resistance in *L. angustifolius* is based on a single dominant gene AnR and was used to breed the resistant cv. Rancher which was released in the United States. The action of the gene is temperature sensitive, becoming progressively less effective from 22 to 28 °C (Wells & Forbes, 1967). This gene was also incorporated into the Australian cv. Illyarrie.

The Australian *L. angustifolius* cultivars Tanjil and Wonga have a higher level of resistance than Illyarrie. More recently, the cv. Kalya has been shown to have a different resistance gene.

Three co-dominant locus specific molecular markers have been identified which are closely linked to the dominant Tanjil resistance gene Lanr1 (Yang et al., 2004) and these are now used for routine marker-assisted selection in Australian breeding programs. Only lines carrying the resistant marker are retained. However, field screening is still conducted on these lines to choose phenotypes with the highest expression of resistance.

An excellent source of resistance has been identified in *L. albus* from a landrace collected in Ethiopia (P27174) and held in the Australian Lupin Collection. The genetic basis of this resistance has not been confirmed but it is being used as a parent in Australian breeding programs.

Resistance in all species is best expressed in stem tissue. High levels of resistance to pod infection have not been identified in any species to date.

Phomopsis stem and pod blight

Ascospores from infected stubbles initiate subcuticular latent infections on stems and pods which develop into dark purplish lesions at or after crop maturity under moist conditions. Secondary conidia produced on defoliated leaves within the growing season can also initiate latent stem and pod infection. There is little published data on variation in the pathogen but Shankar et al. (1995) reported strains specialised to *L. angustifolius*, *L. albus* and *L. luteus* in Australia.

Techniques for screening

Phomopsis stem blight occurs regularly under Australian field conditions, particularly when lupins are grown in close rotation. Pod blight occurs less frequently. Breeders have been able to exploit natural epidemics and have assessed resistance by rating the frequency and intensity of stem lesions on stubble immediately prior to the crop being harvested (Cowling et al., 1987).

Williamson et al. (1991) developed an artificial inoculation, tissue staining and microscopic examination procedure to visualise subcuticular coralloid mycelia which are a latent infection structure. Shankar et al. (1996) standardised the inoculation and incubation procedures as was able to show that breeding lines known to be resistant or susceptible under field conditions had small or large coralloid structures respectively. An ELISA test, based on polyclonal antibodies to *D. toxica*, was developed to distinguish large (susceptible) and small (resistant) pathogen biomass as an alternative to microscopic examination (Shankar et al., 1998).

Sources of resistance

A wild *L. angustifolius* line collected from Spain (P22750) was identified as resistant and used as a parent to produce the first resistant cvs. Gungurru and Merrit (Cowling et al., 1987). A breeding line 75A:258 was shown to have an extremely resistant phenotype which can be traced to a wild parent from Morrocco (P22872). Screening of F1, F2 and F3 families from crosses between 75A:258, Merrit and susceptible cv. Unicrop showed that 75A:258 had a single dominant allele Phr1 which conferred resistance, whereas Merrit carried a different incompletely dominant resistance allelle Phr2 (Shankar et al., 2002). A co-dominant locus specific molecular marker has been produced based on an MFLP polymorphism linked to the Phr1 gene (Yang et al., 2002). A marker for Phr2 is under development.

The European *L. albus* cvs. Ultra and Kiev Mutant and the *L. luteus* cvs. Teo and Motiv 369 have a good resistance to phomopsis stem blight in Australia.

Brown spot

As well as being transmitted by seed, the thick-walled and pigmented conidia of *P. setosa* can survive in soil. Soil-borne spores are rain-splashed and deposit predominantly on the lower leaf surface. Within 2–3 days, necrotic lesions develop and after 2 weeks, infected leaves begin to senesce and defoliate (Yang et al., 1996). Yang and Sweetingham (2002) found that lupin isolates of the pathogen in Australia were quite uniform but that a distinct strain occurs on serradella (*Ornithopus* spp.) that is less pathogenic to lupin. Lupin isolates from Europe and North America are more closely related to the Australian lupin than serradella strain.

Techniques for screening

In Australia, high levels of disease pressure occur where multiple cropping of lupins (on the same field) develops a high population of conidia in the soil (Sweetingham, 1990). Brown spot can be assessed from the six-leaf stage through to flowering. Glasshouse methods of inoculation have been developed. Conidia concentrations of 25,000 ml^{-1}, temperatures in the range 13–17 °C and 18 h leaf wetness post-inoculation give disease expression comparable to natural field infections (Yang et al., 1996). A rapid measure of brown spot severity is to count the number of primary leaves defoliated on the main stem. Rating scales that estimate the percentage of leaf area infected can be used but are much more labour intensive.

Sources of resistance

Yang and Sweetingham (1998) found that *L. luteus* was more resistant than *L. angustifolius* and that *L. albus* was the specie most susceptible to brown spot.

No major genes for resistance to brown spot have been found in *L. angustifolius*, but there are quantitative differences amongst breeding lines. The cv. Myallie has moderate resistance to defoliation (Cowling et al., 1997), the result of a recurrent selection breeding approach to accumulate polygenic resistance.

Pod and seed infection is less in taller lines through escape from rain-splash of soil-borne spores.

Necrotrophic foliar diseases of pea

As ascochyta blight is considered as the most important necrotrophic foliar disease on pea worldwide (Bretag & Ramsey, 2001), this chapter concerns only this disease.

Ascochyta blight of pea (*Pisum sativum* L.) is caused by three related fungal species, commonly referred to as the Ascochyta complex: *Ascochyta pisi* Lib., *Ascochyta pinodes* Berk. & Bloxam (teleomorph: *Mycosphaerella pinodes* Berk. & Bloxam) and *Phoma medicaginis* var. *pinodella*, formerly known as *Ascochyta pinodella* (L.K. Jones) Boerema (Jones, 1927). A teleomorph of *Ascochyta pinodella* has recently been described by Bowen et al. (1997). The teleomorphs may contribute to the development of new pathotypes

or strains of the fungi that may vary in virulence, morphology or cultural characteristics. All three pathogens can be present either together on the pea plant as a complex, or as a single pathogen.

Symptoms develop on all aerial parts of the plant (leaves, stems, pods and seeds) and consist of necrotic lesions that eventually affect seed number and size, leading to substantial yield and seed quality losses (Allard et al., 1993). The lesions caused by *A. pisi* are different from those caused by *M. pinodes* and *P. medicaginis* var. *pinodella*. *A. pisi* causes well-delineated lesions: generally, there are one to five lesions per organ. At the centre of the necrotic lesions, numerous pycnidia are formed. On the stems, this fungus causes deep necrotic lesions which can lead to breaking of stems and death of plant parts above the affected zone. Initially, *M. pinodes* and *P. medicaginis* var. *pinodella* produce small lesions, which appear in the form of numerous flecks. The disease spreads rapidly and foliar infections are severe after the onset of flowering. Leaves with many lesions wither before the lesions become large, especially on the lower portion of the plants. Stem lesions are initiated at the base of the dead leaf and spread above and below that point; with time, they coalesce to encircle the entire lower stem, which generally does not break. All three fungi can cause necrosis on pods that often result in seed infection.

M. pinodes and *P. medicaginis* var. *pinodella* cause footrot and similar symptoms on leaves, stems, pods and seeds (Hare & Walker, 1944).

Choice of appropriate isolates

Choice of isolate is of crucial importance for accurate resistance screenings. When there are specific interactions between pathotypes and host genotypes, the use of the main pathotypes in screening experiments is necessary. When these interactions are non-race specific, the level of aggressiveness is then the criterion for choosing the isolate for the inoculation. Choosing the most aggressive isolate is not always the best, since the level of disease may then be too high to allow the detection of slight differences between genotypes.

Whether or not race-specific interactions are present in the *M. pinodes–P. sativum* pathosystem is still a matter of concern. There are numerous reports on the existence of pathotypes: Ali et al. (1978) differentiated 15 pathotypes in Australia by using 38 host pea differentials; Clulow et al. (1991a) isolated 9 pathotypes and 16 pathotypes related, respectively, to stem and foliar reactions of 9 host lines; Nasir and Hoppe (1991) differentiated 6 pathotypes by using 6 pea differential lines; Xue et al. (1998) grouped 275 isolates into 22 pathotypes by their differential reactions on 21 differential pea genotypes. However, other studies led to different conclusions: Wroth (1998b) studied the pathogenicity of 99 Australian *M. pinodes* isolates and concluded at an absence of specific interaction with pea lines. Onfroy et al. (1999) reported no evidence for pathotypes among 50 French *M. pinodes* isolates. The six genotypes under study differed in their reactions to various isolates of *M. pinodes* and *P. medicaginis* var. *pinodella* but the ranking of the differentials was constant. The contribution of genotype × isolate interactions to variation was very low and differences in pathogenicity were not due to differences in the virulence of the isolates, but rather to resistance/susceptibility differences in the genotypes. There is therefore no definitive evidence for *M. pinodes* pathotypes based on virulence, which seems to be arbitrary and subjective (Knappe & Hoppe, 1995). These inconsistent results may be due to the rating systems used to assess resistance. In studies where the quantitative response is transformed into a more discrete assessment based on resistant and susceptible genotypes, the authors usually conclude that specific interactions are present (Ali et al., 1978; Clulow et al., 1991a; Nasir & Hoppe, 1991, 1997; Xue et al., 1997); whereas, taking into account the entire continuous variation leads to the conclusion of non-specific interactions (Wroth, 1998b; Onfroy et al., 1999).

It has been suggested (Parlevliet, 1979) that in some systems the major part of the genetic variation for resistance can be independent of the pathogen genotype, i.e. race non-specific, but that the remaining minor part could be dependent on the pathogen genotype, thus defining pathotypes and race specificity. This may be the case for the *M. pinodes–P. sativum* system. Nasir and Hoppe (1991), for instance, acknowledged that their proposed pathotype grouping is probably based both on differential differences in virulence and on non-differential differences in aggressiveness. Conversely, Onfroy et al. (1999) concluded that there was no race specificity based on the relative contribution to the total variation of the genotype × isolate interaction compared to the contribution of genotype and isolate main effects. The interaction effect was however significant. It is therefore possible that some assessment methods may be more accurate than others in displaying minor specificity effects.

It is possible furthermore that pathotypes exist that specifically infect stems or leaves. Indeed, Clulow et al. (1991a) insisted that some *M. pinodes* isolates were

able to colonize stems but not the leaves and *vice versa*. Although the disease was always more severe on leaves than on stems, studies by Onfroy et al. (1999) did not confirm these results, probably owing to differences in the methodologies used. This issue still needs to be addressed more precisely.

For *A. pisi*, the existence of true pathotypes was proved by Darby et al. (1985, 1986) who showed that some isolates can produce either a hypersensitive reaction or a typical necrosis on leaves of resistant and susceptible hosts, respectively. These authors proposed the existence of five distinct pathotype groups based on highly significant isolate/genotype interactions.

Techniques for screening

Partial resistance assessments are usually based on slight quantitative differences between cultivars; the methodology used for assessments is therefore a key issue to successful and reproducible results in controlled conditions as well as in the field.

Inoculation techniques must ensure homogeneous disease spread to effectively discriminate among test entries. Artificial inoculation to measure reactions of pea genotypes to *M. pinodes* include methods such as depositing a droplet of a spore suspension either on detached leaflets (Wroth, 1996; Wang et al., 2000) or on stems and leaves directly on the plant (Nasir et al., 1992; Clulow et al., 1992; Wroth, 1998a, 1999), agar discs of mycelium to inoculate stems (Clulow et al., 1991a), immersion of seeds in a spore suspension (Nasir & Hoppe, 1991; Clulow et al., 1991a), spray inoculation with a spore suspension on specific leaves (Zimmer & Sabourin, 1986) or the spraying inoculation of the whole plant (Ali et al., 1978; Nasir et al., 1992; Nasir & Hoppe, 1997; Onfroy et al., 1999; Prioul et al., 2003). The inoculation methodology can be organ specific when reactions seem to differ between leaves and stems for instance (Clulow et al., 1992).

Screening methodology should also define the choice of isolate based on its pathogenicity and adequate inoculum pressure to display resistance behaviour. On the basis of their results, Onfroy et al. (1999) suggested the use of the most highly aggressive isolate for screening would not be the best choice.

Field inoculation methods usually attempt to simulate natural infection depending on cropping systems. Among the possible techniques are the use of infected pea stubble (Ali et al., 1978; Xue et al., 1996; Wroth, 1998a; Wroth & Khan, 1999; Wang et al., 2000), spreading of barley or sugar beet grains infected with various isolates (Tivoli et al., 1996; Kraft et al., 1998; Prioul et al, 2004), or allowing a natural epidemic of the fungus to develop (Bretag, 1991; Timmerman et al., 2002; Tar'an et al., 2003a,b). Alternatively, spray inoculations of spore suspensions of mixed isolates (Xue et al., 1997; Boros & WieWiora, 2004) have also been used in the field.

Rating symptoms of disease has varied in the criterion addressed. Some of the disease scales are based on the number of spots on the leaves and the percentage of leaf area diseased together with the length and percentage of necrosis on stems or footrot symptoms. These variables have been used quantitatively (Zimmer & Sabourin, 1986), as semi-quantitative (Clulow et al., 1991a; Nasir and Hoppe, 1991, 1997; Tivoli et al., 1996; Xue et al., 1996; Onfroy et al., 1999; Wroth, 1998a,b; Timmerman et al., 2002) or as discrete, considering resistant, intermediate and susceptible types (Ali et al., 1978). In some cases of semi-quantitative variables, the area under the disease progress curve (AUDPC) was calculated by plotting mean disease severity against time (Wroth & Khan, 1999; Xue & Warkentin, 2001; Prioul et al., 2003, 2004). Other disease scales are based on the measurement of percentage of severely infected plants in the canopy (Kraft et al., 1998). Alternatively, scales were developed that integrate percentage of height with a certain level of disease (Tivoli, 1994; Prioul et al, 2004), or percentage of height and disease severity together (Baranger & Tivoli, 1998). Obviously, authors have developed different ways to assess resistance. Partial resistance scoring requires the development of relevant criterion to assess slight differences between lines (Xue & Warkentin, 2001), and some criterion might be more relevant than others depending on the environmental conditions, plant growth stage and the degree of disease pressure.

Generally, for each screening test, authors have used only one isolate but some have used mixtures of isolates. For example, in a seedling test for responses to *M. pinodes* infection, Wroth (1998a) used a spore suspension mixture in equal proportions of three isolates and Prioul et al. (2004), under field conditions, used a mixture of infected barley lots infected separately with four single-spore isolates of *M. pinodes* which differed only in their aggressiveness.

Up to now, resistance shown at early growth stages in the growth chamber have been ineffective in identifying high levels of resistance in field plot conditions. This is probably due to differing disease pressure between the growth chamber and the field, but also to strong interactions of disease severity in the field with

agronomic traits such as maturity, lodging or plant height. This does not necessarily mean that these resistance sources are useless to improve resistance at the adult stage in the field. Indeed, growth chamber experiments are more suitable than field assessments to measure intrinsic resistance levels, highly correlated to genetic resistance alleles, whereas field assessments measure these genetic effects together with strong interactions of these effects with environmental conditions. Assessment methodologies in the field could therefore focus on either an analysis of these interactions in order to evaluate what their part is in the variation, or try to get rid of them by carrying out resistance assessments for instance on stacked pea lines (without lodging), or among maturity, lodging or plant height groups (Baranger et al., 1997). This should lead to better correlations with the growth chamber results.

Sakar et al. (1982) gave special attention to techniques of screening peas for resistance to *P. medicaginis* var. *pinodella*. The techniques used included footrot screening by seed inoculation and foliar screening by spraying innoculum on the leaves and showed that for 93 pea genotypes a lack of correlation between footrot and foliar disease scores, indicating that different genetic factors control reactions to the two phases of the disease.

Techniques used to screen for resistance to *A. pisi* are the same as for both *M. pinodes* and *P. medicaginis* var. *pinodella*. Wang et al. (2000) used an excised leaf-assay technique and Darby et al. (1986) used spraying of spore suspension on whole plants in the greenhouse.

Sources of resistance

In the *M. pinodes – P. sativum* pathosystem, most authors acknowledge that there is no complete resistance available up to now, and that known sources show moderate susceptibility or moderate resistance, thus describing partial resistance, and reduced pathogen development (Clulow et al., 1991a; Nasir & Hoppe, 1991; Knappe & Hoppe, 1995; Xue et al., 1996; Tivoli & Onfroy, 1997; Kraft et al., 1998). Table 3 gives an example of the type of result that can be obtained in a screening experiment for resistance under controlled conditions, using a 0–5 scoring scale (Onfroy et al., 1999). Obviously, the line FP is susceptible, but shows reproductively lower scores than the other lines, and is therefore qualified as partially resistant. These authors, by comparing pea resistance for *M. pinodes* and *P. medicaginis* var. *pinodella*, concluded that the six genotypes tested differed in their reaction to various isolates of both fungi but the ranking of the differentials was constant towards isolates within each pathogen, and between both pathogens (Table 3). The high correlation between resistance observed for both these fungi confirms previous observations by Knappe and Hoppe (1995).

Table 3. Mycosphaerella pinodes (*Mp*) and *Phoma medicaginis* var. *pinodella* (*Pm*) disease severity on pea leaves, assessed 10 days after inoculation in a growth chamber following a 0–5 disease scale (Onfroy et al., 1999)

Pea genotype	FP	DP	Melrose	JI 252	Solara	JI 296
Mp disease severity	2.3 d	2.6 c	2.7 c	3.0 b	3.0 b	3.9 a
Pm disease severity	0.8 c	1.0 c	1.1 c	1.4 b	1.0 c	2.6 a

Means followed by the same letter in a row are not significantly different ($P = 0.05$) according to Newman–Keuls test.

Some authors have studied factors that may be responsible for reducing the rate of infection in partially resistant lines. Nasir and Hoppe (1991), by spraying on leaves and stems, showed that the pea lines or cultivars PF 35323, Danto, Rondo, Bohatyr, WAV-F 750 and PF 31905 can be selected as differentials. Nasir et al. (1992) have shown after droplet inoculations that symptoms appear later and that measured lesion expansion is smaller in a partially resistant line (WavF502) than in a susceptible control (Katrin). Light- and electron microscopy studies after spray inoculation showed that vesicle-like structures at infection sites and that penetrating hyphaes were produced at lower frequencies in the partially resistant pea line. Zimmer and Sabourin (1986) have considered disease development over time, assessing differences in the percentage leaf area infected at the seedling stage among four field pea cultivars. They concluded that cultivars Century and Trapper carry a rate reducing resistance that cultivars Tara and Triumph do not.

From the general behaviour of each genotype (AUDPC for example), some sources of resistance were identified. In some cases, genotypes were examined for components of partial resistance to ascochyta blight. Xue and Warkentin (2001) described several components: leaf area with symptoms, stem area with symptoms, pod area with symptoms and percentage of seed infection. Differences among pea lines were found in all components: some of them are resistant for one or several components.

In several countries, these sources of resistance were used to increase the level of pea resistance and/or to study the genetics of ascochyta blight resistance. In United Kingdom, Clulow et al. (1991b), by crossing resistant lines JI 97 and JI 1089 with a susceptible

line demonstrated that leaf and stem resistance were controlled by different genes. A source of resistance found in *P. fulvum* JI 1006 used as the pollen parent was evaluated in crosses with *P. sativum* cv. Wirrega, a commercial cultivar, using wild type *P. sativum* JI 252 as a bridging cross (Wroth, 1998a). Timmerman et al. (2002) characterized the genetics of resistance and identified molecular markers from families produced from a cross between resistant breeding line 3148-A88 and susceptible cultivar Rovar. A population of 135 recombinant inbred lines, derived from the cross between DP (partially resistant) and JI 296 (susceptible), was genotyped with morphological and molecular markers (Prioul et al., 2004). Tar'an et al. (2003a,b), studied molecular markers in a cross between Carneval (resistant) and MP 1401 (susceptible).

For *A. pisi*, Darby et al. (1986), proposed a set of lines nominated as standard differential hosts including lines having hypersensitive reaction to lines having a very susceptible reaction. The existence of both differential and non-differential components was observed. The non-differential variation appeared quantitative: JI 181 and JI 1097 showed moderate and high resistance to all isolates (Table 4). Cousin (1992) has defined seven physiological races and six differential hosts. Iqbal et al. (2001) observed a difference of behaviour of the tested lines at seedling and flowering stages. At the flowering stage, among the 52 lines tested, three lines (86P117-5, 88P022 and 88P0-6-29) were highly resistant, 15 were resistant, 13 were tolerant and all other were susceptible.

Necrotrophic foliar diseases on chickpea

Chickpea is grown mainly in developing countries (South Asia, West Asia, the Middle East, North Africa and South America) and constitutes the main source of protein in the diet of poor populations. Ascochyta blight, caused by *Ascochyta rabiei* (Pass.) Lab. [teleomorph: *Didymella rabiei* (Kovachevski) v. Arx], is the most important fungi disease of chickpea. It affects above-ground parts of the plants causing 100% yield loss in some situations (Nene, 1984). BGM caused by *B. cinerea* is of lesser importance but it is also a widespread foliar disease problem.

Ascochyta blight lesions on leaves are light brown or tan with a dark brown margin, they tend to be elongated and dark brown with a dark margin on stems, while on pods they are generally circular. Pycnidia are observed within the lesions as small dark bodies and usually in concentric rings. Seed transmission of the disease and the presence of the sexual stage are main factors responsible for extension of infestation to new areas (Kaiser, 1997). Although the sexual stage has been reported only in few regions in the world, it does not mean that its presence is not generalized in all areas infested by the disease (Kaiser, 1997). The strategy applied by farmers in the Mediterranean region and some other countries to avoid development of heavy blight infestations is to delay sowing the crop to spring. However, lower yields are often obtained due to a shortened life cycle and less-favourable conditions for chickpea production. Over the past 30 years the international centres (ICRISAT and ICARDA) and national programmes throughout the world have concentrated on development of chickpea germplasm and cultivars with resistance to ascochyta blight. A major goal was to develop ascochyta blight resistant chickpea germplasm that would be adapted to winter sowing in order to benefit more from the favourable conditions for crop development.

Choice of appropriate isolates

Pathogenicity-defined isolates used for inoculation is the most critical factor for successful controlled environment screening. The use of defined isolates improves repeatability, facilitates comparisons of results among different laboratories, and can reveal genetic mechanisms of resistance (Udupa & Baum, 2003; Cho et al., 2004).

The widely varying reports of races and pathotypes of *A. rabiei* (Vir & Grewal, 1974; Qureshi & Alam, 1984; Reddy & Kabbabeh, 1985; Udupa et al., 1998; Bayaa et al., 2004; Porta-Puglia et al., 1996; Jamil et al., 2000; Chongo & Gossen, 2001; Chongo et al., 2004; Maden et al., 2004) have been problematic in the design and interpretation of results from screening. Classification of isolates into three pathogenicity groups (I, II and III) by Udupa et al. (1998) has become the prevailing view (Table 5). That view classifies isolates into pathotypes according to their level of virulence. There is some discrepancy in the classification between both scales, which could be attributed to the fact that Linear Infection Index (LII) scale takes into consideration only the lesions observed on stems; however, the 1–9 disease severity scale considers symptoms on the whole plant.

Standardization of pathogenicity tests and the use of a consensus differential set of germplasm lines and cultivars tested under similar conditions for disease development are needed in order to critically compare

Table 4. Range of responses resulting from interactions between *Pisum* and *Ascochyta pisi* (Darby et al., 1986)

Standard reaction line	JI 1097	JI 423	JI 181	JI 228	JI 250	JI 403
Reaction class (to race C)	0	1	2	3	4	5
Visible symptoms	Very occasional hypersensitive reaction	Hypersensitive reactions, slight wilting of inoculated leaves. No lesions or stem infection	Occasional small leaf or stipule lesions. Tissue browning at the leaf and petiole junction. No stem lesions	General leaf and stipule infection but no stem lesions	General leaf and stipule infection. Stem lesions but no collapse of plants	General leaf, stem and stipule infection. Considerable leaf wilt. Stems usually become brittle and death of infected plants is frequent

Table 5. Classification of isolates tested against a chickpea differential set in growth chamber and evaluated by 1–9 scale of Reddy and Singh (1984) and the Linear Infection Index of Riahi et al. (1990)

Scale of scoring	High-virulence group[a]	Average-virulence group[b]	Low-virulence group[c]
Reddy and Singh (1984) scale (DS)	Ar46-7, Ar3-1, Ar11-8, Ar44-5, Ar6-4, Ar13-1, Ar45-1, Ar10-2	Ar4-4, A41-1, Ar43-2, Ar1-1	Ar2-3, Ar62-1, Ar61-1, Ar56-6, Ar7-1
Riahi et al. (1990) scale (LII)	Ar4-4, Ar13-1	Ar46-7, Ar11-8, Ar44-5, Ar45-1, A41-1	Ar2-3, Ar6-4, Ar3-1, Ar7-1, Ar43-2, Ar61-1, Ar62-1, Ar1-1, Ar10-2, Ar56-6

[a]Isolates gave susceptible disease reaction on 7 or 8 chickpea lines.
[b]Isolates gave susceptible disease reaction on 3–6 chickpea lines.
[c]Isolates gave susceptible disease reaction on 0–2 chickpea lines.

evaluation results. Most importantly, virulent isolates and standardized procedures need to be used in selection programs to identify chickpea lines resistant to ascochyta blight.

Techniques for screening

Controlled environment screening. Inoculum of selected isolates of *A. rabiei* should be prepared using standard media such as chickpea agar or V8 juice agar (Nene & Reddy, 1987; Chen et al., 2005). Cultures are routinely incubated at 20–22 °C for 12–14 days followed by inoculum preparation and standardization. The optimum inoculum concentration should be tested on different host genotypes. The ideal inoculum level is the lowest inoculum concentration that causes sufficient disease on a majority of host genotypes that will enable discrimination among the lines in the trial. Inoculum concentrations that are too high can cause overwhelming disease that can obscure differences among the lines.

Temperature and relative humidity are two critical factors in controlled inoculations. Temperature is easier to control than humidity. For ascochyta blight, we have found that a high level of relative humidity during the first 24 h after inoculation is very critical. Even under growth chamber conditions, where relative humidity can be controlled, additional devices are needed to maintain relative humidity for successful infection (Udupa & Baum, 2003; Chen et al., 2005). A mini-dome technique for pathogenicity assay was developed and is in use at Pullman, USA (Chen & Muehlbauer, 2003). The technique consists of inoculating 2-week-old seedlings by spraying with a conidia suspension until run-off and immediately followed by covering the inoculated plants with inverted translucent plastic cups to form mini-domes for 24 h. The purpose of the mini-domes is to form a uniformly high level of humidity to promote disease development. This technique works equally well in growth chambers as well as in the greenhouse, where relative humidity generally cannot be precisely controlled. This technique should be very useful in areas where growth chamber facilities are not readily available because it is inexpensive, portable and expandable to fit any size of experiment. The results obtained with the mini-dome assay were highly correlated with field data (Chen et al., 2005).

In glasshouse tests, temperature should be maintained between 15 and 22 °C during the experiment, and 3–4-week-old plants be inoculated to run-off with a spore suspension that has been adjusted to a concentration of 2×10^5 spores ml^{-1}. Immediately after inoculation, the plant should be covered for 48 h to guarantee high humidity, helping the penetration of the fungus and development of infection. This can be very effectively done using the mini-dome procedure as mentioned earlier (Chen & Muehlbauer, 2003). The material should be scored at the beginning of disease development, usually 8–10 days after inoculation and at weekly intervals thereafter until termination of the experiment.

Field screening. Field screening is an inexpensive means of identifying resistant genetic material, provided that disease pressure is sufficient to distinguish between entries. Field screening techniques for ascochyta blight resistance in chickpea were developed by Singh et al. (1981) and were based on the methods applied earlier by Labrousse, Luthra et al. and Sattar (cited by Nene & Reddy, 1987). Screening should be carried out in areas where the prevailing weather conditions are conducive to the development of disease and preferably where natural inoculum is abundant. The procedure consists of planting susceptible checks every two or four tested entries, scattering infected debris collected in previous season, maintaining high

humidity through sprinkler irrigation and, if needed, spraying the test entries with a spore suspension of a virulent isolate or mixture of isolates of *A. rabiei*. Also, it is important to include a resistant check in order to compare resistance of test entries with known resistant material.

Infested debris should be applied to the disease nursery when the plants are 6–8 cm tall. Infested barley, chickpea or rye seeds can also be used to inoculate the disease nursery in the absence of infested chickpea debris (Tivoli et al., 1996; Haware et al., 1995; Chongo & Gossen, 2001). Appropriate arrangement of plant materials to be screened in the nursery is important. Sufficient replications should be used and arranged in blocks that have susceptible chickpea genotypes interspaced throughout the nursery to promote uniform disease development and spread across the nursery. Sites chosen for disease nurseries should have a history of ascochyta blight occurrence without complicating factors of other stress problems.

Temperature and atmospheric humidity play an important role in the development of ascochyta blight (Trapero-Casas & Kaiser, 1992a,b). Sprinkler irrigation can be important in supplementing relative humidity; however, during hot and dry conditions, supplemental irrigation may not be effective in promoting the disease. Under such conditions, irrigation after sunset can be effective in maintaining high relative humidity and promoting disease development. Due to the association between plant age and disease susceptibility, Porta-Puglia et al. (1994) stated that it is necessary to expose material to the disease at all crop growth stages even after the susceptible check may have been destroyed.

When it is necessary to inoculate using a spore suspension it should be done late in the afternoon or early in the evening at concentration 3×10^5 spores ml^{-1}. In the absence of rain, sprinkler irrigation should also be applied to improve disease development and spread. Scoring of the test entries should be done early and at least twice during the season. Scoring is most often done at the beginning of flowering and at the mid podding stage.

In vitro screening. Strange et al. (2004) have researched the prospect of a laboratory test using solanapyrone A for screening purposes. The toxin is produced by the fungus and has been shown to have an important role in the expression of blight disease symptoms.

Disease scoring procedures

Almost all foliar diseases of chickpea caused by necrotrophic fungi are quantitative in nature. That means that disease severity is usually continuous and there is no clear delineation between resistant and susceptible reactions. Consequently, there is no simple resistant and susceptible scoring. Disease scoring procedures must reflect this continuous nature. A number of scoring procedures have been developed for assessing disease severity of foliar diseases of chickpea.

Parametric methods. Parametric methods involve direct measurements of lesion sizes (Riahi et al., 1990), direct counts of number of lesions (Riahi et al., 1990), percentage of infected leaves (Chen et al., 2005), and percent affected area (Lichtenzveig et al., 2002). The direct counts or measurements have the advantage of being objective, but they are time consuming. Estimating the percent affected area requires special training and is subjective. Therefore, this procedure can only be approximate to certain ranges, which render it similar to scaled rating. Computer-aided image analysis may help alleviate the problem.

Non-parametric methods. Non-parametric methods usually are scaled scoring methods. The most commonly used scaling method for ascochyta blight is the 1–9 rating scale developed by Reddy and Singh (1984). This 1–9 rating method is based on diseased tissues relative the whole plant. This method has been widely used for studying ascochyta blight and has proven its usefulness (Jan & Wiese, 1991; Haware et al., 1995; Navas-Cortés et al., 1998). A 1–9 rating scale was also developed for BGM (Tripathi & Rathi, 2000).

The 1–9 scale for ascochyta blight on chickpea was converted to disease severity values using the formula:

$$\text{Disease severity } (\%) = \frac{\Sigma(\text{number of plants in a category values} \times \text{category value}) \times 100}{\text{total number of plant} \times \text{maximum category value}}.$$

Strange et al. (2004) reported that appreciation of the reaction by using the 1–9 scale was much better at the extremes of the scale than at the middle, where large standard deviations were observed. Other scales have been used but not extensively such as 0–5 scale (Khan et al., 1999) or 0–11 scale of Horsfall–Barrat (Chongo & Gossen, 2001).

When scores are made in different times, area under disease progress curve (AUDPC) could also be determined and used for discriminating between susceptible and resistant entries. AUDPC effectively changes integer scores taken at different times during the season into a single measure of disease severity. Tekeoglu et al. (2000) and Lichtenzveig et al. (2002) used AUDPC for evaluation of ascochyta blight of chickpea and Prioul et al. (2003) recommended the utilization of this scale for genetic studies for ascochyta blight of pea. However, Tekeoglu et al. (2000) could not show an advantage for AUDPC over the simple 1–9 scale of scoring.

Riahi et al. (1990) proposed LII for evaluation of ascochyta blight of chickpea. This quantitative scale takes into consideration the number of lesions and their length on the stem as well the total length of the stems. It is time consuming, not practical for screening large collections, and does not take into consideration the symptoms observed on leaves. The relationship between the 1–9 rating scale and percent leaf infection are highly correlated with each other (Figure 1). It is recommended for genetic studies conducted in controlled conditions and also for identifying specific molecular markers.

An attempt to use image analysis software to determine diseased area showed that the system could be recommended for growth chamber or greenhouse tests. Analyses of the symptoms taken in the field were less precise and more difficult to assess (Strange et al., 2004). A pictorial scale (1–9) to help pathologists and breeders in scoring ascochyta of chickpea has been produced (Strange et al., 2004).

The disease scoring methods have implications in statistic analysis. Data obtained with either a parametric method or the non-parametric 1–9 rating scale can be used in Analysis of Variance (ANOVA), when the data sets meet the assumptions (a random sample from each population, each population with normal distribution and all the populations having the same variance) of ANOVA. However, if the variance is unequal, data obtained with the parametric methods can be transformed to equalize the variance before implementing ANOVA. A non-parametric procedure would be preferable over ANOVA when unequal variance is found in data obtained with any of the non-parametric scoring methods.

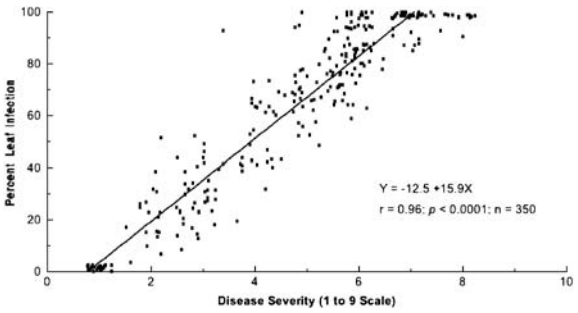

Figure 1. Correlation between the non-parametric 1–9 rating scale and the parametric percent leaf infection in chickpea.

There is no single scoring method that is best for all situations. Consequently, researchers should decide the most appropriate methods depending on the research objectives, familiarity and comfort level with a particular method and available resources. In many situations, different scoring methods yield highly correlated data and lead to the same or similar conclusions. We have tested the relationship between the 1–9 rating scale and percent leaf infection, and found that these two methods are highly correlated with each other (Figure 1). Using a parametric scoring method, Lichtenzveig et al. (2002) concluded that resistance to ascochyta blight of chickpea is conditioned by quantitative trait loci with some major effect, supporting the conclusion derived from using the 1–9 rating scale (Santra et al., 2000; Tekeoglu et al., 2000; Udupa & Baum, 2003; Cho et al., 2004).

Sources of resistance

Breeding cultivars and germplasm with resistance to necrotrophic fungi has intensified in recent years and become a major factor in production, particularly in developed countries such as the United States, Canada, Australia and Turkey. Due to the scarcity of sources of resistance to ascochyta blight in cultivated chickpea, a small number of resistant accessions have been widely used in the crossing programs most notably ILC482, ILC3279, FLIP84-92C and FLIP84-79C (Singh, 1987; Reddy & Singh, 1984, 1992; Malhotra et al., 1996). Efficient and accurate screening procedures for identification of selections with improved resistance have been a critical factor in cultivar development.

In the continued search for additional genes for resistance to necrotrophic fungal pathogens, wild species have received increased attention. The wild *Cicer* species comprise 8 annuals and 34 perennials but only *C. reticulatum* and *C. echinospermum* are crossable to the cultivated *C. arietinum*. Crosses of *C. reticulatum* with the cultigen have been fully fertile and there are no barriers to gene flow between the two species.

However, crosses of *C. echinospermum* with *C. arietinum* have a high degree of sterility in the F_1 and succeeding progenies. Crosses with other wild species including the perennials, *C. songaricum* and *C. anatolicum*, have generally met with failure; and, thus far, no viable hybrids have been obtained. The crossable wild species do not appear to be a source of resistance to ascochyta blight or Botrytis grey mold; however, increased attention towards *C. echinospermum* as a resistance source may have merit.

Necrotrophic foliar diseases of faba bean

The common foliar diseases on faba bean (*Vicia faba* L.) are Ascochyta blight and Chocolate spot. Ascochyta blight, caused by the fungus *Ascochyta fabae* Speg., is worldwide distributed (Gaunt, 1983). Yield losses of about 40% are common, but losses can be as high as 90% in susceptible cultivars (Hanounik, 1980), particularly under wet and cool weather conditions. Chocolate spot is a destructive leaf disease of faba bean that can reduce yields by more than 60% (Sundheim, 1973; Hanounik, 1981; Mabsoute & Saadaoui, 1996), particularly in humid regions.

Ascochyta blight

A. fabae infects all above-ground plant parts including seeds, and the damage includes reduction in photosynthetic area, lodging following stem girdling, pod and seed abortion, and seed infection. Infected seeds are the main source of inoculum, where the fungus is borne in the testa only (Pritchard et al., 1989). Infected crop debris and volunteer plants may also have a role in the pathogen spreading. Disease control through crop rotation, clean seeds, and chemical treatment has not been completely effective and the development of resistant cultivars is widely recognized as the most efficient method of control.

Choice of appropriate isolates

Physiological specialization between pathogen isolates and host genotypes has been described in the *A. fabae*–faba bean pathosystem (Kharbanda & Bernier, 1980; Ali & Bernier, 1985; Hanounik & Robertson, 1989; Rashid et al., 1991a; Kohpina et al., 1999; Avila et al., 2004) suggesting the existence of races, what is however, still controversial. Hanounik and Robertson (1989) found that eight isolates from Syria could be grouped into four races based on their interaction with four faba bean genotypes selected as differential set. Later, Rashid et al. (1991a) proposed a new differential set composed by eight faba bean lines. Like this, they were able to group 10 isolates into 7 races of the pathogen. Both works could not be compared, since they did not share the same plant material. Standardisation of the inoculation and evaluation methods is needed. Besides, the development of a unique differential set would allow the identification of races around the complete distribution zone.

The existence of races is an important consideration in breeding programs for disease resistance and in developing disease management systems, making necessary the search for additional sources of resistance. In addition, *Didymella fabae* Jellis & Punith., the teleomorph of *A. fabae*, has recently been discovered in UK (Jellis & Punithalingam, 1991) and reported in several countries such as Spain (Rubiales & Trapero, 2002) and Australia and might be present in other countries, as compatible mating types have been found also in debris from Algeria, Lebanon and Syria (Kaiser, 1997). Sexual reproduction allows new virulence combination and as a consequence the pathogen may respond over time to selection exerted by the introduction of host resistance genes.

Techniques for screening

Field evaluations. Field screening is commonly used to evaluate faba bean germplasm against *A. fabae*. It can be done by sowing in naturally infested fields, i.e., those fields where inoculum pressure is sufficient. Alternatively, it is possible to inoculate the field trial to ensure a homogeneous distribution of the disease avoiding disease escape. This can be done by spraying plants with a conidial suspension of *A. fabae* (1–2×10^5 conidia ml^{-1} is enough), by spreading infested barley seeds (10 g m^{-2}) or debris at sowing early after plant emergence (Maurin & Tivoli, 1992). The accessions should be inoculated after sunset in order to take advantage of the darkness and the high humidity of the night to ensure high and uniform levels of infection. Relative humidity can be increased by irrigating the plots with micro-sprinklers several times a day. A susceptible control should be distributed throughout the trial. A first assessment of disease development should be made early, on young plants with about the fourth-fifth leaves completely expanded. A second assessment can be done at flowering and a third on mature pod-bearing plants.

Several scales can be used. Disease severity scale (DS) is based on the percentage of symptomatic area

of the whole plant, while the qualitative scale Infection Type (IT) (Rashid et al., 1991a) is based on the lesion size and the presence or not of pycnidia. This last scale is rather quick but not suitable for field observations when few lesions are present that could not easily be found. The evaluation could result in underestimation of rate-reducing resistance types resulting in low DS but where the few lesions visible are well developed. This scale might be very useful in growth chamber studies complemented with DS ratings.

Other scales try to enclose both criteria. Thus, the 0–9 disease scoring recommended by ICARDA (Bernier et al., 1984) is a combination of lesion type, lesion frequency and extent of damage, being more complete than IT alone, and also quick, which makes it more adequate to be used in large-scale breeding programs. However, we recommend to slightly modify the ICARDA scale including among low ratings those representing relative little amount of well-formed lesions to avoid discarding useful sources of resistance. The Disease Index calculated as the mean of the scores per node in a 0–4 scale (Maurin & Tivoli, 1992) also takes into account both amount and type of lesions, as does the scale of ICARDA. Thus, this index would have efficiently identified the resistance of those lines reducing DS but allowing development of some big lesions. This index is too time consuming for a field screening of a big collection, but a simplification of such index evaluated directed as the visual average per plot could be of great help in mass screenings and selection activities.

Ascochyta blight pod infection can lead to seed transmission, thus being of importance. Several screenings for *A. fabae* resistance have been performed looking for reduction of pod infection (Lockwood et al., 1985; Maurin & Tivoli, 1992; Sillero et al., 2001). Sillero et al. (2001) described a pod infection rated on a 0–5 scale, where 0: no symptoms of pod infection and 5: >30% of pod area covered with lesions and some pods broken because of the lesions. Finally, incidence of infected seed can be assessed by assaying a sample of 50 seeds per plot. The seeds are surface sterilized and then plated out on potato dextrose agar. After 7 days incubation under near-ultra-violet light (12-h photoperiod) at room temperature, the number of colonies of *A. fabae* are counted. Under dry conditions, selection for resistance should be made on foliage and stems but not on pods, as there might be escape infection due to dry and warm conditions at this plant stage. The negative correlation of pod infection with days to flowering and with length of straw often reported (Jellis et al., 1985; Lockwood et al., 1985; Sillero et al., 2001) indicates that both factors might also determine escape to pod infection.

Growth chamber evaluation. Growth chamber evaluation allows performing screening avoiding environmental effect. It is recommended to sow 5–10 plants per accession in at least three consecutive repeats. Susceptible and resistant checks should be included. Plants can be inoculated by spraying a suspension of conidia of the appropriate isolate of *A. fabae* (5×10^5 spores ml^{-1}). Plants should be incubated for 24 h in darkness and 100% relative humidity, and then kept at 20 °C with a 14/10-h day/night photoperiod cycle in a growth chamber. Disease development can be evaluated 15 days after inoculation with any of the scales indicated previously. In addition to this, components of resistance such as lesion size, latency period and spore production can be measured for a more accurate description of partial resistance. Growth chamber and greenhouse trials are as effective as field experiments for identifying resistant genotypes (Tivoli et al., 1987; Sillero et al., 2001).

Detached leaf test. The pathogenic variability described in this pathosystem by several authors, suggests the necessity of genotype evaluations against different isolates of the pathogen. This problem may be handled using detached-organ tests. This method allows obtaining a detailed description of a genotype by testing of individual leaves of single plants against several pathogen isolates. Similarly, since the reactions of stems and leaves often differ (Kohpina et al., 1999; Avila et al., 2004), the use of a stem segment may also be necessary. The detached-leaf assay allows evaluating germplasm in a uniform environment. Kohpina et al. (2000) developed detached-organ techniques effective in the testing of faba bean genotypes for their response to ascochyta blight. According to their work, plants would need to grow for 3–5 weeks after sowing to allow a good discrimination of disease response. Young leaves (one to three from the top of the plant) were the most suitable for disease response studies using for inoculation a suspension of 5×10^4 spores ml^{-1} (Kohpina et al., 2000). The most informative criterion for discriminating the level of disease resistance was the proportion of leaves infected. Detached organs were maintained in good conditions for more than 10 days, which is the period required for the appearance of symptoms.

Table 6. Sources of resistance to major necrotrophic pathogens in faba bean

Disease	Sources of resistance	References
Ascochyta blight	IB19, Bulldog, Banner, Buccaneer, IB7CS, IB18	Bond and Pope (1980)
	IB18-1/3	Lockwood et al. (1985)
	BPL 74, BPL 460, BPL 471, BPL 472, BPL 646, BPL 818, BPL 2485, ILB 1814	Hanounik and Robertson (1989)
	15025-2, 15035-1, 15041-2, ACK-1-21, ACK-1-9, ACK 2-2, ERF-3-14	Rashid et al. (1991a,b)
	29H, 29M, 972bc	Maurin and Tivoli (1992)
	SU-R 40, SU-R 5/13	Ondrej (1993)
	BPL 230, BPL 266, BPL 365, BPL 465, ILB 752, L8 3118, L8 3120, L8 3124, L8 3125, L8 3127, L8 3129, L8 3136, L8 3142, L8 3149, L8 3151, L8 3155, L8 3156, L8 2001, L8 31818-1, Quasar, Line 224	Reviewed by Bond et al. (1994)
	V-1220, V-494, V-175, V-47, V-165, V-1122, V-46	Sillero et al. (1991)
	ILB 1414, ILB 6561	Bayaa et al. (2004)
	Ascot (selection from a land race from island of Naxos)	Ramsey et al. (1995)
Chocolate spot	BPL 666, ILB 938, Maris Bead	Jellis et al. (1985)
	BPL 710, BPL 1179	Hanounik (1982)
	BPL 1196, BPL 1179-1, BPL 261, BPL 266, BPL 274, BPL 470, BPL 1055, BPL 1058, BPL 1278, BPL 1390, BPL 1543, BPL 1544, BPL 1547, BPL 1548, BPL 1550, BPL 1763, BPL 1821, ILB 1814	Hanounik and Maliha (1986)
	ILB 2282, ILB 3025, ILB 3026, ILB 3027, ILB 3028, ILB 3029, ILB 3030, ILB 3031, ILB 3033, ILB 3056, ILB 3079, ILB 3091, ILB 3104, ILB 3105	Hanounik and Robertson (1988)
	BPL 110, BPL 112, BPL 2282, L82003, L82009, L83114, Zhehiang 41 LAO, Qi Dou No.2, Lu-Xiao-Li-Zhong	Reviewed by Bond et al. (1994)
	FRY98-3, FRY98-15, FRY98-38, FRY98-48, FRY98-52	Rhaïem et al. (2001)
	PF 44, LPF 237, LPF 05, LPF 113	Rhaïem et al. (2002)
	ILB 1414, ILB 6561	Bayaa et al. (2004)
	FRYM167, FRYA58	Bouhassan et al. (2004)

Sources of resistance (Table 6)

Very few resistance sources to *A. fabae* were available by the 1970s, but since then several sources have been identified and used in breeding programs, although none resulted in complete resistance (Bond & Pope, 1980; Tivoli et al., 1988; Hanounik & Robertson, 1989; Rashid et al., 1991a,b; Maurin & Tivoli, 1992; Ondrej, 1993; Bond et al., 1994; Sillero et al., 2001). Complete resistance was observed in INRA line 29H (Maurin et al., 1993). Different methods of disease assessment have been used by different authors under different environmental conditions and plant development stages, which makes it hard to compare the results. Resistance has either been described as a reduction in the amount of lesions (Ondrej, 1993), a reduction in the infection type (Rashid et al., 1991a) or both a reduction in disease severity and infection type (Hanounik & Robertson, 1989; Maurin & Tivoli, 1992). Reduced infection can also be due to morphological plant features that facilitate disease escape such as length of straw (Jellis et al., 1985; Lockwood et al., 1985; Maurin & Tivoli, 1992). Combination of both low tannin content in the seeds and resistance to diseases is of major interest in faba bean breeding programs. However, relation has been found between tannin content and resistance to pathogenic soil-borne fungi (Helsper et al., 1994; Kantar et al., 1996) suggesting that tannins may have fungicidal properties which protect seeds and seedlings during germination and emergence. Such association was not observed with resistance to *A. fabae* (Helsper et al., 1994, Sillero et al., 2001).

Chocolate spot

Chocolate spot can be caused both by *Botrytis fabae* Sardina and *B. cinerea* Pers., but *B. fabae* is more important being more aggressive in the field (Harrison, 1988). Severe outbreaks are most common in the Nile delta, near rivers in China, rainy coastal areas of the Mediterranean, and the more oceanic climate of western France and western UK. Losses are determined by the transition from non-aggressive to aggressive forms of the disease during flowering and early pod set. Although chemical control may provide partial

protection, it is costly, reduces the crops profitability, and is harmful to the environment. Therefore, breeding of resistant cultivars by developing appropriate and effective methods and techniques of screening is a priority to control the disease and an appropriate strategy to promote the development of sustainable agriculture.

Choice of appropriate isolates

Only varying levels of quantitative resistance have been reported. The use of a very aggressive isolate would not allow the expression of small differences of reactions between genotypes (Parlevliet, 1983). Application of a single virulent isolate inoculum, instead of a mixture of isolates with a wide range of variation for virulence, can avoid confusion between vertical resistance and horizontal resistance (Parlevliet, 1983; Hanounik & Maliha, 1986).

The existence of races of *B. fabae* is a matter of concern and an important factor to take into account in the development of a breeding program. Several authors have reported differences in virulence among isolates (Hutson & Mansfield, 1980; Hanounik & Maliha, 1986) and Hanounik & Maliha (1986) reported the first evidence of races in *B. fabae* populations. They grouped 12 isolates of the pathogen into 4 groups, which they named as races 1, 2, 3 and 4. Their work indicated that these races were more common in Europe than in the Middle East and it was suggested that other races of *B. fabae* may also exist. Besides, the confirmation of race existence is needed from tests of all sources of inoculum on the putative differentials in the same environment (Bond et al., 1994).

Techniques for screening

Field screening. Faba bean disease screening nurseries should normally be established in humid areas where environmental conditions favour maximum disease development. Planting date is an important factor in disease development. In general, chocolate spot is favoured by early planting. A local susceptible check should be grown as frequently as every third row to help spread the disease and develop a uniform disease pattern throughout the nursery. Best results are obtained when artificial inoculations are done on cloudy rainy days, particularly at sunset. To produce and multiply inoculum stored sclerotia or fresh leaves or stems showing symptoms of the aggressive stage of chocolate spot (dark brown blackish lesions) should be surface sterilized in a 0.5% sodium hypochlorite solution for 1–2 min, plated on PDA medium, and incubated at room temperature (20–25 °C). Cultures obtained should be incubated at room temperature for about 10 days on faba bean leaf extract medium (Leach & Moore, 1966) on which large amount of conidia are formed. Conidia are then harvested and diluted with sterile tap water until a spore suspension containing about $4–5 \times 10^5$ ml^{-1} spores is obtained that is sprayed over plants about 2 months after sowing. Inoculated nurseries could be covered with polythene sheets, supported by metal frames. Polythene sheets should be removed on sunny days and replaced after sunset. However, on continuous rainy days, as long as the leaf surface remains wet there is no need to cover the nursery with polythene sheets. High humidity can be maintained by sprinkling the plants with a fine vapour of water several times a day, until the symptoms appear extensively on the susceptible lines.

Disease symptoms can be scored weekly on the basis of a 1–5 visual scale (Bouhassan et al., 2004), being a combination of lesion type, lesion frequency and extent of damage, with 1: no symptoms or very small spots; 2: very small and discrete lesions; 3: some coalesced lesions with some defoliation; 4: large coalesced sporulating lesions, 50% defoliation, some plants dead; 5: extensive lesions on leaves, stems and pods, severe defoliation, heavy sporulation, blackening and death of more than 80% of the plants. ICARDA suggests a 1–9 scale based on similar principles, where 1: no lesions or few small brown, non-sporulating specks, covering up to 1% of leaf surface; 3: few small, discrete, brown, circular, non-sporulating lesions (2–3 mm in diameter) covering 1–2% of leaf surface; 5: lesions common (3–5 mm in diameter), some coalesced, covering 2–5% of leaf surface, with some defoliation and very poor sporulation; 7: Large coalesced irregular blackish sporulating lesions that cover 5–10% of leaf surface, average defoliation, flower drop, and some dead plants; 9: extensive large coalesced heavily sporulating lesions, covering more than 10% of the leaf surface, with severe defoliation, stem girdling, and death of great majority of plants.

A single assessment at about 3 weeks after inoculation in the field can be sufficient to rank the genotypes for their reaction to the disease (Hanounik & Robertson, 1988; Bouhassan et al., 2004), but sequential observations and calculation of AUDPC values is recommended to study the disease evolution. This may be of importance under fluctuating climatic conditions influencing the development of the disease. In addition, the stage 3 weeks after inoculation may coincide with a different seasonal period under different environmental conditions depending on planting date.

Detached leaf test in the laboratory. This is a simple and rapid laboratory test designed to double check results from chocolate spot screening nurseries in the field. Fully-expanded leaflets of similar physiological age should be collected from about the eighth node position and laid flat on a moistened filter paper laid on sterile benches. The cut end of each leaflet petiole is covered with moistened cotton to maintain leaves at maximum turgor. The upper side of the leaves is inoculated with 1.5 ml of a spore suspension containing about 5×10^5 ml^{-1} spores of *B. fabae*, one droplet on each half of each leaflet. The benches are then covered with polythene sheets and left at room temperature ($20 \pm 2\,^{\circ}$C) for 5–6 days till disease is assessed. A 1–4 scale can be used, where 1: no infection or very small flecks (1–25% necrosis); 2: necrotic flecks with few small lesions (26–50% necrosis), and very poor sporulation; 3: medium coalesced lesions (51–75% necrosis) with intermediate sporulation; 4: large coalesced lesions (76–100% necrosis) with abundant sporulation. Disease progress can be recorded by scoring every day the disease symptoms using a nine-class scale (Gondran, 1977) for 9 days. Additionally, diameter of emerging lesions can be measured during this period and spore production can be determined 11 days after inoculation by putting leaflets in a fixed volume of sterile water and estimating the number of spores per leaflet using a Malassez haemocytometric cell.

Detached leaf tests usually provide similar results as field tests for highly susceptible and highly resistant genotypes, but there is no good agreement for accessions with intermediate levels of resistance (Tivoli et al., 1986; Bouhassan et al., 2004). Nevertheless, this test can be very useful for undertaking the first screening of a large number of lines and thereby eliminating highly susceptible genotypes before conducting costly field tests. The difference in reaction of faba bean genotypes to chocolate spot by the two screening tests is undoubtedly due to the fact that each method permits expression of some particular components of resistance more clearly than others (Tivoli et al., 1986). Bouhassan et al. (2004) found that a combination of field disease score with lesion diameter measured in detached leaf test improved discrimination between accessions, which suggests a potential use of this test in the study of the components of partial resistance.

Sources of resistance (Table 6)

Resistance to chocolate spot of different cultivars varies, but is not high. High resistance has been found in related species such as *V. narbonensis*, but faba bean can not cross with any other *Vicia* species (Gondran, 1977). Substantial levels of resistance in faba bean were not reported till the 1980s (Hanounik & Robertson, 1988). The most resistant germplasm has generally originated from the Andean region of Columbia and Ecuador (Hanounik & Robertson, 1988; Bond et al., 1994). In fact, the most consistently resistant accessions developed by ICARDA (lines BPL 710 and BPL 1179) are originated from close to a mountainous region in Ecuador. Recently, Bouhassan et al. (2004) detected significant differences among genotypes for reactions to the disease in the field. However, no complete resistance was observed. The lines FRYM167 and FRYA58 seem to have a high level of partial resistance (Bouhassan et al., 2004).

General conclusion

This review shows that, except for two cases, namely *A. pisi* on pea and *A. fabae* on faba bean, *resistance of grain legumes to necrotrophic diseases is partial*, highly dependent on environmental factors, inoculum pressure and/or plant stage. Environmental factors, such as temperature, humidity, light and wind have an effect both on the pathogen cycle and on the growth and receptivity of the host plant, and their main or combined effects may affect disease development from one cultivar to another. Too high inoculum concentrations may make it more difficult to detect differences between cultivars. The best conditions to display resistance may therefore be intermediate pressure, marginally favourable for the pathogen. Plant physiological and growth stage are also factors that can give rise to various conditions that can be more or less favourable to pathogen development. Resistance behaviours observed in the field are for instance often related to maturity types. The effect of the physiological age of the plant tissues on the expression of resistance is a critical issue in some pathosystems. Partial resistance expression, as a cycle rate-reducing phenomenon, is highly dependent on these conditions and screening techniques should focus on developing conditions reducing these genotype $\times$ environment interactions, both in the field or under controlled conditions.

For all necrotrophic foliar pathogens considered previously, the knowledge of *pathogenicity variability is a key issue* to choose appropriate isolates to screen for resistance. The usefulness of identified resistance sources for genetic studies or breeding programs depends highly on their ability to resist in a wide range of conditions or towards the most widespread pathotypes

in a cropping region for instance. Pathogenicity reports are sometimes controversial, but for most necrotrophic pathogens in grain legumes (except for *A. pisi* and *A. fabae*), variation is quantitative and the variability based more on aggressiveness than on true virulence. Already developed within countries, pathogenicity studies should therefore enlarge to between countries by sharing consensus on differential host sets, isolates and standardized inoculation and evaluation procedures to give an insight into the actual differences in pathogen diversity between countries or cropping regions. Whether a resistance source is wide range or specific to a pathotype is crucial to its subsequent use in identifying genes for marker-assisted selection and breeding.

Screening techniques are very diverse, mostly aiming at selecting resistant lines (sometimes at eliminating the most susceptible) through the use of semi-quantitative disease scales applied at the whole plant level or the canopy level. Uniformity is a main concern, under controlled conditions techniques as well as in the field (use of susceptible checks as infection raws). It is therefore of crucial importance to establish standardised environmental and inoculation conditions that are best suited to precisely measure the relative incidence of cultivar and environmental effects as well as their interaction. Some techniques aim at understanding in more details how resistance expresses, from one organ to the other, or at dissecting resistance into more quantitative criteria which are dependent on phases of the disease cycle (penetration, disease appearance, symptoms extension, sporulation, etc.). This task often necessitates more precise and labour-intensive screening techniques, often under controlled conditions, sometimes on detached organs. The so-called components of resistance, originating from different sources, could be combined to raise the overall level of resistance not only at the seedling stage, but also at the adult stage by slowing down the different stages of the infection cycle. These strategies will give an insight into the potential interest of pyramiding into breeding lines different genes controlling different resistance components to ensure a better efficiency of quantitative resistance in the field.

Identified sources of resistance towards a specific pathogen are sometimes very diverse, some being close to the breeding ideotype, other looking more like exotic accessions unadapted to cropping conditions in terms of plant architecture, yield potential, etc. The knowledge of the passport data for accessions, of the pedigree for breeding lines, of their genetic proximity through molecular studies, and of the way they express resistance (range towards pathogenicity, organ specificity, components) should be addressed to better understand their interest in terms of pyramiding genes into breeding lines, possibly through marker-assisted selection following genetic studies and QTL or gene identification. The potential use of non-specific resistance, the effect of numerous genes acting on distinct resistance parameters exerting less pressure on the pathogen, is thought to make the available resistance more durable over time (Parlevliet, 2002).

The model legume Medicago truncatula should be a great tool to identify and characterise resistance genes to necrotrophic pathogens of grain legumes in the future. Recently, this annual medic has gained a status of model legume because of its interesting characteristics for both classical and molecular genetic studies, including diploid nature, self-fertile and small genome size, which resulted in the development of large genetic, genomic and sequencing resources in this species (Cook, 1999). This species is closely related to important economically grain legumes species, including pea (Doyle et al., 1996) and conserved synteny already has been found (Gualtieri et al., 2002). This suggests that genetic information from the model plant could be useful to understand the genetics of pea resistance. To date, although reports have indicated large genetic variation between *M. truncatula* ecotypes (Bonnin et al., 1996), no systematic search for sources of disease resistance has been performed to explore this natural germplasm variability. There are many necrotrophic diseases of annual *Medicago* species, among which Phoma black stem disease, caused by *Phoma medicaginis*, anthracnose (also known as Colletotrichum crown rot) caused by *Colletotrichum trifolii*, Leptosphaerulina leaf and stem spot (*Leptosphaerulina trifolii*, also known as *L. briosiana*), Pseudopeziza leaf spot (*Pseudopeziza medicaginis*), Stemphylium leaf spot (*Stemphylium botryosum* and *S. vesicarium*), Stagonospora leaf spot (*Stagonospora meliloti*), only *Phoma medicaginis* being common to other grain legumes. Some sources of resistance to these pathogens are known (M. Barbetti, unpublished results). On the other hand, *M. truncatula* is also known as being a host of some pathogenic fungi of grain legumes, including pea, faba bean, chickpea and lentil, and showing variation towards these pathogens (Ellwood et al., 2004; Moussart, unpublished results). Both from being a host of numerous necrotrophic aerial pathogens and from being a host of other grain legumes necrotrophic pathogens, *M. truncatula* may be of particular interest to dissect resistance

to these pathogens. Genetic and genomic resources in this species will help to boost genetical analysis in grain legumes species and therefore contribute to a better understanding of the structure and function of resistance genes to necrotrophic fungi in legumes.

References

Agrios, G.N., 2004. Plant pathology, 5th edn., 952 pp. Academic Press, Harcourt Brace Jonanovich, Publishers.

Ahmad, M., A.C. Russell & D.L. McNeil, 1996. Identification and genetic characterization of different resistance sources to ascochyta blight within the genus *Lens*. Euphytica 97: 311–315.

Ahmed, S. & S.P.S. Beniwal, 1991. Ascochyta blight of lentil and its control in Ethiopia lentil. Tropical Pest Man 37: 368–373. CAB Abstracts.

Ahmed, S.K. & R.A.A. Morrall, 1995. Population changes in *Ascochyta fabae* f. sp. *lentis* over 15 years of lentil cultivation in Saskatchewan. In: Proceedings of the 2nd European Conference on Grain Legumes, 9–13 July, Copenhagen, Denmark, pp. 76–77.

Ali, S.M., 1995. Register of Australian grain legume cultivars. *Lens culinaris* (lentil) cv. Northfield. Aust J Exp Agric 35: 1181–1182.

Ali, F.H. & C.C. Bernier, 1985. Evaluation of components of resistance to *Ascochyta fabae* in faba beans (*Vicia faba*). Phytopathology 75: 962.

Ali, S.M., L.F. Nitschke, A.J. Dubé, M.R. Krause & B. Cameron, 1978. Selection of pea lines for resistance to pathotypes of *Ascochyta pinodes*, *A. pisi* and *Phoma medicaginis* var. *pinodella*. Aust J Agric Res 29: 841–849.

Allard, C., L. Bill & G. Touraud, 1993. L'anthracnose du pois. Revue bibliographique et synthèse. Agronomie 3: 5–24.

Anderson, K.L., 2003. Pathogenic variation and host specificity of *Colletotricum truncatum* from lentil. M.Sc. Thesis, University of Saskatchewan, Saskatoon, Canada.

Anderson, K.L., L. Buchwaldt, G. Chongo, B.D., Gossen, R.A.A. Morrall & P.G. Pearse, 2000a. Diseases of lentil in Saskatchewan 1999. Can Plant Dis Surv 80: 96–98.

Anderson, K.L., L. Buchwaldt, J. Sillero & B.D. Gossen, 2000b. Host range of *Colletotrichum truncatum* from lentil. Can J Plant Pathol 22: 180.

Andrahennadi, C.P., 1994. Genetics and linkage of isozyme markers and resistance to seedborne *Ascochyta* infection in lentil. M.Sc. Thesis, University of Saskatchewan, Saskatoon, Canada. As cited in Ye et al. (2002).

Andrahennadi, C.P., A.E. Slinkard & A. Vandenberg, 1996. Ascochyta resistance in lentil. LENS Newslett 23: 5–7.

Avila, C.M., Z. Satovic, J.C. Sillero, D. Rubiales, M.T. Moreno & A.M. Torres, 2004. Isolate and organ-specific QTLs for ascochyta blight resistance in faba bean. Theor Appl Genet 108: 1071–1078.

Bahl, P.N., S. Lal & B.M. Sharma, 1993. An overview of the production and problems of lentil in South Asia. In: W. Erskine & M.C. Saxena (Eds.), Lentil in South Asia, pp. 1–10. ICARDA, Aleppo, Syria.

Baranger, A. & B. Tivoli, 1998. Involvement of partial resistance in the genetic control of diseases. The case of ascochyta blight resistance in pea. In: AEP (Ed.), Proceedings of the 3rd European Conference on Grain Legumes, 15–19 November 1998, Valladolid, Spain, pp. 39–45.

Baranger, A., B. Tivoli, C. Onfroy., G. Morin & J. Le Guen, 1997. Estimation of variation for resistance to *Mycosphaerella pinodes* in field-grown pea lines. In: Proceedings of the Third International Food Legume Research Conference, 22–26 September, Adelaide, Australia, p. 169.

Bayaa, B. & W. Erskine, 1998. Diseases of lentil. In: D.J. Allen & L.M. Lenné (Eds.), The Pathology of Food and Pasture Legumes, pp. 423–472. Wallingford, UK.

Bayaa, B., W. Erskine & A. Hamdi, 1994. Response of wild lentil to *Ascochyta fabae* f. sp. *lentis* from Syria. Genet Crop Evol 41: 6–65.

Bayaa, B., M. Kabakebji, S. Khalil, S. Kabbabeh & K. Street, 2004. In: Proceedings of the 5th European Conference on Grain Legumes. Legumes for the benefit of agriculture, nutrition and the environment: Their genomics, their products, and their improvement, 7–11 June, Dijon, France, p. 308.

Bernier, C.C., L. Buchwaldt & R.A.A. Morrall, 1992. Screening for resistance to anthracnose in lentil (*Lens culinaris*). In: Proceedings of the 1st European Conference on Grain Legumes, 1–3 June, Angers, France, pp. 37–38.

Bernier, C.C., S.B. Hanounik, M.M. Hussen & H.A. Mohamed, 1984. Field manual of common bean diseases in the Nile Valley. ICARDA Inform Bull 3: 40.

Bond, D.A., G.J. Jellis, G.G. Rowland, J. Le Guen, L.D. Robertson, S.A. Khalil & L. Li-Juan, 1994. Present status and future strategy in breeding faba beans (*Vicia faba* L.) for resistance to biotic and abiotic stresses. Euphytica 73: 151–166.

Bond, D.A. & M. Pope, 1980. *Ascochyta fabae* on winter beans (*Vicia faba*): Pathogen spread and variation in host resistance. Plant Pathol 29: 59–65.

Bonnin, I., T. Huguet, M. Gherardi, J.M. Prosperi & I. Olivieri, 1996. High level of polymorphism and spatial structure in a selfing plant species, *Medicago truncatula* Gaertn. using RAPDs markers. Am J Bot 83: 843–855.

Boros, L. & B. WieWiora, 2004. Evaluation of varietal susceptibility in pea to *Mycosphaerella pinodes*. In: AEP/INRA (Ed.), Proceedings of the 5th European Conference on Grain Legumes, 7–11 June 2004, Dijon, France, p. 316.

Bouhassan, A., M. Sadiki & B. Tivoli, 2004. Evaluation of a collection of faba bean (*Vicia faba* L.) genotypes originating from the Maghreb for resistance to chocolate spot (*Botrytis fabae*) by assessment in the field and laboratory. Euphytica 135: 55–62.

Bowen, J.K., B.G. Lewis & P. Matthews, 1997. Discovery of the teleomorph of *Phoma medicaginis* var. *pinodella* in culture. Mycol Res 101: 80–84.

Bretag, T.W., 1991. Epidemiology and control of Ascochyta blight of field peas. Ph.D. Thesis, La Trobe University, Victoria, Australia, pp. 1–297.

Bretag, T.W. & M.A. Materne, 1998. Lentil: Effect of seeding rate on foliar disease and grain yield. In: J. Hyett (Ed.), Summary of Field Research in the Wimmera 1998, pp. 35–36. Department of Natural Resources and Environment, Horsham, Victoria.

Bretag, T.W. & M. Ramsey, 2001. Foliar diseases caused by fungi: *Ascochyta* spp. In: J.M. Kraft & F.L. Pfleger (Eds.), Compendium of Pea Disease and Pests, 2nd edn., pp. 24–28. APS Press.

Buchwaldt, L., K.L. Anderson, R.A.A. Morrall, B.D. Gossen & C.C. Bernier, 2003. Identification of lentil germplasm resistant to *Colletotrichum truncatum* and characterization of two pathogen races. Phytopathology 94: 236–243.

Buchwaldt, L., R.A.A. Morrall, G. Chongo & C.C. Bernier, 1996. Windborne dispersal of *Colletotrichum truncatum* and survival in infested lentil debris. Phytopathology 86: 1193–1198.

Buchwaldt, L., A. Vandenberg & C.C. Bernier, 1995. Selection in lentil for anthracnose resistance and seed characteristics. In: Proceedings of the 2nd European Conference on Grain Legumes, 9–13 July, Copenhagen, Denmark, pp. 90–91.

Chen, W., C.L. Coyne, T.L. Peever & F.J. Muehlbauer, 2004. Characterization of chickpea differentials for pathogenicity assay of ascochyta blight and identification of chickpea accessions resistant to *Didymella rabiei*. Plant Path 53: 759–769.

Chen, W. & F.J. Muehlbauer, 2003. An improved technique for virulence assay of *Ascochyta rabiei* on chickpea. Int Chickpea Pigeonpea Newslett 10: 31–33.

Cho, S., W. Chen & F.J. Muehlbauer, 2004. Pathotype-specific genetic factors in chickpea (*Cicer arietinum* L.) for quantitative resistance to ascochyta blight. Theor Appl Genet 109: 733–739.

Chongo, G. & C.C. Bernier, 1999. Field and growth chamber evaluation of components of partial resistance to *Colletotrichum truncatum* in lentil. Can J Plant Pathol 21: 58–63.

Chongo, G. & C.C. Bernier, 2000a. Disease incidence, lesion size, and sporulation in *Colletotrichum truncatum* as influenced by lentil genotype and temperature. Can J Plant Pathol 22: 236–240.

Chongo, G. & C.C. Bernier, 2000b. Effects of host, inoculum concentration, wetness duration, growth stage, and temperature on anthracnose of lentil. Plant Dis 84: 544–548.

Chongo, G. & B.D. Gossen, 2001. Effect of plant age on resistance to *Ascochyta rabiei* in chickpea. Can J Plant Pathol 23: 358–363.

Chongo, G., B.D. Gossen & C.C. Bernier, 2002. Infection by *Colletotrichum truncatum* in resistant and susceptible lentil genotypes. Can J Plant Pathol 24: 81–85.

Chongo, G., B.D. Gossen, L. Buckwaldt, T. Adhikari & S.R. Rimmer, 2004. Genetic diversity of *Ascochyta rabiei* in Canada. Plant Dis 88: 4–10.

Chowdhury, M.A., C.P. Andrahennadi, A.E. Slinkard & A. Vandenberg, 2001. RAPD and SCAR markers for resistance to ascochyta blight in lentil. Euphytica 118: 331–337.

Clulow, S.A., B.G. Lewis & P. Matthews, 1991a. A pathotype classification for *Mycosphaerella pinodes*. J Phytopathol 131: 322–332.

Clulow, S.A., P. Matthews & B.G. Lewis, 1991b. Genetical analysis of resistance to *Mycosphaerella pinodes* in pea seedlings. Euphytica 58: 183–189.

Clulow, S.A., B.G. Lewis & P. Matthews, 1992. Expression of resistance to *Mycosphaerella pinodes* in *Pisum sativum*. Plant Pathol 41: 362–369.

Cook, D., 1999. *Medicago truncatula*: A model in the making! Curr Opin Plant Biol 2: 301–304.

Cousin, R., 1992. Les protéagineux. Le pois. In: A. Gallais & H. Bannerot (Eds.), Amélioration des espèces végétales cultivées. Objectifs et critères de sélection, INRA Editions, Vol. 768, pp. 172–188.

Cowling, W.A., 1996. Plant breeding for stable agriculture: Presidential address 1994. J R Soc West Aust 79: 183–194.

Cowling, W.A., B.J. Buirchell, I. Frencel, S. Koch & J.M. Neves-Martins, 1999. International evaluation of resistance to anthracnose in lupin. In: Proceedings of the 9th International Lupin Conference, Klink, Germany, pp. 16–22.

Cowling, W.A., J. Hamblin, P.McR. Wood & J.S. Gladstones, 1987. Resistance to Phomopsis stem blight in *Lupinus angustifolius* L. Crop Sci 27: 648–652.

Cowling, W.A., M.W. Sweetingham, D. Dieperveen & B. Cullis, 1997. Heritability of resistance to brown spot and root rot of narrow-leafed lupins caused by *Pleiochaeta setosa* (Kirchn.) Hughes in field experiments. Plant Breed 116: 341–345.

Darby, P., B.G. Lewis & P. Matthews, 1985. Inheritance and expression of resistance to *Ascochyta pisi* Lib. In: P.D. Hebblethwaite, Health & Dawkins (Eds.), The Pea Crop – A Basis for Improvement. Butterworths, London.

Darby, P., B.G. Lewis & P. Matthews, 1986. Diversity of virulence within *Ascochyta pisi* and resistance in the genus *Pisum*. Plant Pathol 35: 214–223.

Davidson, J.A., M. Materne, K.D. Lindbeck, M. Krysinska-Kaczmarek & D. Hartley, 2004. Understanding Botrytis grey mould of lentils in Australia and identifying useful resistance. In: 5th European Grain Legumes Conference, 7–11 June, Dijon, France, pp. 83–84.

Doyle, J.J., J.L. Doyle, J.A. Ballenger & J.D. Palmer, 1996. The distribution and phylogenetic significance of a 50 kb chloroplast DNA inversion in the flowering plant family Leguminosae. Mol Phylogenet Evol 5: 429–438.

Ellwood, S., L. Kamphuis, N. D'Souza, R.M. Nair & R.P. Oliver, 2004. Resistance to fungal necrotrophs in *Medicago truncatula*. In: 5th European Grain Legumes Conference, 7–11 June, Dijon, France, p. 343.

Erskine, W. & M.C. Saxena, 1993. Problems and prospects of stress resistance breeding in lentil. In: K.B. Singh & M.C. Saxena (Eds.), Breeding for Stress Tolerance in Cool-Season Food Legumes, pp. 51–62. Wiley, New York.

Erskine, W., M. Tufail, A. Russell, M.C. Tyagi, M.M. Rahman & M.C. Saxena, 1994. Current and future strategies in breeding lentil for resistance to biotic and abiotic stresses. Euphytica 73: 127–135.

Ford, R., S. Banniza, W. Photita & P.W.J. Taylor, 2004. Morphological and molecular discrimination of anthracnose caused by *Colletotrichum truncatum* from Canadian lentil. Australasian Plant Pathol 33: 559–569.

Ford, R., E.C.K. Pang & P.W.J. Taylor, 1999. Genetics of resistance to ascochyta blight (*Ascochyta lentis*) of lentil and the identification of closely linked RAPD markers. Theor Appl Genet 98: 93–98.

Gaunt, R.E., 1983. Shoot diseases caused by fungal pathogens. In: P.D. Hebblethwaite (Ed.), The Faba Bean, pp. 463–492. Butterworths, London.

Gibson, R.J., 1994. An investigation into the epidemiology and control of anthracnose (*Colletotrichum truncatum*) of lentil in Manitoba. M.Sc. Thesis, University of Manitoba, Winnipeg, Canada.

Gondran, J., 1977. Résistance de la vesce de Narbonne et de la féverole à *Botrytis fabae*. Revue Marocaine, Science Agronomique Véterinaire, pp. 288–295.

Gondran, J., R. Bournoville & C. Duthion, 1994. Identification of diseases, pest and physical constraints in white lupin. INRA Editions Versailes, France, 47 pp.

Gossen, B.D. & R.A.A. Morrall, 1983. Effects of ascochyta blight on seed yield and quality of lentils. Can J Plant Pathol 5: 168–173.

Gualtieri, G., O. Kulikova, E. Limpens, D.J. Kim, D.R. Cook, T. Bisseling & R. Geurts, 2002. Microsynteny between pea and *Medicago truncatula* in the SYM2 region. Plant Mol Biol 50: 225–235.

Hanounik, S., 1980. Effect of chemical treatments and host genotypes on disease severity/yield relationships of Ascochyta blight in faba beans. FABIS Newslett 2: 50.

Hanounik, S., 1981. Influence of Ronilan on the severity of chocolate spot and yield of faba bean. Faba Bean Inform Serv Newslett 3: 50–51.

Hanounik, S.B., 1982. Resistance in faba beans to chocolate spot. FABIS Newslett 5: 24–26.

Hanounik, S.B. & N. Maliha, 1986. Horizontal and vertical resistance in *Vicia faba* to chocolate spot caused by *Botrytis fabae*. Plant Dis 70(8): 770–773.

Hanounik, S.B. & L.D. Robertson, 1988. New sources of resistance in *Vicia faba* to chocolate spot caused by *Botrytis fabae*. Plant Dis 72: 696–698.

Hanounik, S.B. & L.D. Robertson, 1989. Resistance in *Vicia faba* germplasm to blight caused by *Ascochyta fabae*. Plant Dis 73: 202–205.

Hare, W.W. & J.C. Walker, 1944. Ascochyta diseases of canning pea. Wisconsin Res Bull 150: 1–31.

Harrison, J.G., 1988. The biology of *Botrytis* spp. on Vicia beans and chocolate spot disease – A review. Plant Pathol 37: 168–201.

Haware, M.P., H.A. Van Rheenen & N.S.S. Prasad, 1995. Screening for ascochyta blight resistance in chickpea under controlled environment and field conditions. Plant Dis 79(2): 132–135.

Helsper, J.P.F.G., A. Van Norel, K. Burgermeyer & J.M. Hoogendijk, 1994. Effect of the absence of condensed tannins in faba beans (*Vicia faba*) on resistance to foot rot, ascochyta blight and chocolate spot. J Agric Sci 123: 349–355.

Hussain, S., M. Nasir & T. Bretag, 2000. Lentil germplasm evaluation for resistance against ascochyta blight caused by *Ascochyta lentis*. Pakistan J Agric Res 16: 118–121.

Hutson, R.A. & J.W. Mansfield, 1980. A general approach to the analysis of mechanisms of pathogenicity in *Botrytis – Vicia faba* interactions. Physiol Plant Pathol 17: 309–317.

Iqbal, S.M., A. Bakhsh & R.A. Malik, 1990. Identification of resistant sources to ascochyta blight in lentil. LENS Newslett 17: 26–27.

Iqbal, S.M., S. Hussain & B.A. Malik, 1992. In vitro evaluation of fungicides against *Botrytis cinerea* of lentil. LENS Newslett 19: 49–51.

Jamil, F.F., N. Sarwar, M. Sarwar, J.A. Khan, J. Geistlinger & G. Khal, 2000. Genetic and pathogenic diversity within *Ascochyta rabiei* (Pass.) lab. populations in Pakistan causing blight of chickpea (*Cicer arietinum* L.). Physiol Mol Plant Pathol 57: 243–254.

Jellis, G.J., G. Lockwood & R.G. Aubury, 1985. Phenotypic influences on the incidence of infection by *Ascochyta fabae* in spring varieties of faba beans (*Vicia faba*). Plant Pathol 34: 347–352.

Jellis, G.J. & E. Punithalingam, 1991. Discovery of *Didymella fabae* sp. nov., the teleomorph of *Ascochyta fabae*, on faba bean straw. Plant Pathol 40: 150–157.

Jones, L.K., 1927. Studies of the nature and control of blight, leaf and pod spot, and footrot of peas caused by species of Ascochyta. NY State Agric Exp Station Bull 547: 1–45.

Kaiser, W.J., 1997. Inter- and international spread of ascochyta pathogens of chickpea, faba bean, and lentil. Can J Plant Pathol 19: 215–224.

Kaiser, W.J., R.M. Hannan & J.D. Rogers, 1994. Factors affecting the growth and sporulation of *Ascochyta fabae* f. sp. *lentis*. Plant Dis 78: 374–379.

Kantar, F., P.D. Hebblethwaite & C.J. Pilbeam, 1996. Factors influencing disease resistance in high and low tannin *Vicia faba*. J Agric Sci 127: 83–88.

Kapoor, S., G. Singh, A.S. Gill & B. Singh, 1990. Lentil lines resistant to ascochyta blight. LENS Newslett 17: 26–28.

Karki, P.B., 1993. Plant Protection of Lentil in Nepal. In: W. Erskine & M.C. Saxena (Eds.), Lentil in South Asia, pp. 187–193. ICARDA, Aleppo, Syria.

Kelley, T.G., P. Parthasarathy Rao & H. Grisko-Kelley, 1997. The pulse economy in the mid-1990s: A review of global and regional developments. In: R. Knight (Ed.), Proceedings IFLRC-III: Linking Research and Marketing Opportunities for the 21st Century, pp. 1–29. Kluwer Academic Publisher, Dordrecht, The Netherlands.

Khan, M.S.A., M.D. Ramsey & E.S. Scott, 1999. Host range studies with an Australian isolate of *Ascochyta rabiei*. Australasian Plant Pathol 28(2): 170–173.

Kharbanda, P.D. & C.C. Bernier, 1980. Cultural and pathogenic variability among isolates of *Ascochyta fabae*. Can J Plant Pathol 2: 139–142.

Khare, M.N., B. Bayaa & S.P.S. Beniwal, 1993. Selection methods for disease resistance in lentil. In: K.B. Singh & M.C. Saxena (Eds.), Breeding for Stress Tolerance in Cool-Season Food Legumes, pp. 107–121. Wiley, New York.

Knappe, B. & H.H. Hoppe, 1995. Investigations on the resistance of peas (*P. sativum* L.) towards *Ascochyta pinodes* and *Phoma medicaginis* var. *pinodella*. In: AEP (Ed.), Proceedings of the 2nd European Conference on Grain Legumes, 9–13 July 1995, Copenhagen, Denmark, pp. 86–87.

Kohpina, S., R. Knight & F.L. Stoddard, 1999. Variability of *Ascochyta fabae* in South Australia. Australasian J Agric Res 50: 1475–1481.

Kohpina, S., R. Knight & F.L. Stoddard, 2000. Evaluating faba beans for resistance to ascochyta blight using detached organs. Australasian J Exp Agric 40: 707–713.

Kraft, J.M., B. Dunne & S. Armstrong, 1998. A search for resistance in peas to *Mycosphaerella pinodes*. Plant Dis 82: 251–253.

Kucharan, M., S. Banniza & B. Vandenberg, 2003. Evaluation of lentil varieties for resistance to Botrytis gray mould. In: Proceedings of Pulse Field Days, 6–7 January 2003, Saskatoon, Canada, p. 89.

Lichtenzveig, J., D. Shtienberg, H.B. Zhang, D.J. Bonfil & S. Abbo, 2002. Biometric analyses of the inheritance of resistance to *Didymella rabiei* in chickpea. Phytopathology 92: 417–423.

Lindbeck, K.D., T.W. Bretag & M.A. Materne, 2003. Breeding for resistance to Botrytis grey mould in Australian Lentil. In: 8th International Congress on Plant Pathology, Christchurch, New Zealand, p. 291.

Lindbeck, K.D., M.A. Materne, J.A. Davidson, L. McMurray & K. Panagiotopoulos, 2002. Lentil Disease Management Strategy for Southern Region GRDC. Pulse Australia and state departments extension article.

Lockwood, G., G.J. Jellis & R.G. Aubury, 1985. Genotypic influences on the incidence of infection by *Ascochyta fabae* in winter-hardy varieties of faba bean (*Vicia faba*). Plant Pathol 34: 341–346.

Mabsoute, L. & E. Saadaoui, 1996. Maladies cryptogamiques de la fève au Maroc. In: W. Bertenbreiter & M. Sadiki (Eds.), The rehabilitation of Faba Bean, pp. 137–146. Proc. 1er séminaire du résau maghrébin de recherche sur fève, 24–27 May 1995, Rabat, Marocco.

Maden, S., F.S. Dolar, I. Babaliogullu, H. Bayraktar & F. Demirci, 2004. Determination of pathogenic variability of *Ascochyta rabiei*

and reactions of chickpea cultivars against the pathotypes in Turkey. In: AEP (Ed.), 5th European Conference on Grain Legumes & 2nd International Conference on Legume Genomics and Genetics, p. 307 [book of abstracts].

Malhotra, R.S., K.B. Singh, H.A. van Rheenen & M. Pala, 1996. Genetic improvement and agronomic of chickpea with emphasis on the Mediterranean region. In: N.P. Saxena, M.C. Saxena, C. Johansen, S.M. Virmani & H. Harris (Eds.), Adaptation of Chickpea in the West Asia and North Africa, ICRISAT/ICARDA, pp. 217–232.

Materne, M.A., T. Bretag, L. McMurray, S. Nitschke & K. Lindbeck, 2002. Genetic variability in lentil for resistance to Botrytis grey mould (*Botrytis* spp.). In: Proceedings of the 12th Australasian Plant Breeding Conference, 15–20 September 2002, Perth, Australia, pp. 809–811.

Maurin, N., J.P. Gourret & B. Tivoli, 1993. Histopathology of interaction between *Ascochyta fabae* and *Vicia faba*: Comparison of susceptible and resistant reactions. Agronomie 13: 921–927.

Maurin, N. & B. Tivoli, 1992. Variation in the resistance of *Vicia faba* to *Ascochyta fabae* in relation to disease development in field trials. Plant Pathol 41: 737–744.

McKenzie, B.A., G.D. Hill, J.G.H. White, G. Meijer, G. Sikken, A. Nieuwenhuyse & A.G. Kausar, 1986. The effect of sowing date and population on yield of lentils (*Lens culinaris* Medik.). Proc Agron Soc New Zealand 16: 29–33.

Morrall, R.A.A., 1988. A new disease of lentil induced by *Colletotrichum truncatum* in Manitoba. Plant Dis 72: 994.

Morrall, R.A.A., 1997. Evolution of lentil diseases over 25 years in Western Canada. Can J Plant Pathol 19: 197–207.

Nasir, M. & T. Bretag, 1997a. Prevalence of *Ascochyta fabae* f. sp. *lentis* on lentil seed from Victoria, Australia. Australasian Plant Pathol 26: 117–120.

Nasir, M. & T. Bretag, 1997b. Pathogenic variability in Australian isolates of *Ascochyta lentis*. Australasian Plant Pathol 26: 217–220.

Nasir, M. & T. Bretag, 1998. Reactions of lentil accessions from 25 different countries to Australian isolates of *Ascochyta lentis*. Genet Res Crop Evol 45: 297–299.

Nasir, M. & H.H. Hoppe, 1991. Studies on pathotype differentiation within *Mycosphaerella pinodes* (Berk. & Bloxam) Vestergren, a component of the Ascochyta-disease-complex of peas (*Pisum sativum* L.). Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 98: 619–626.

Nasir, M. & H.H. Hoppe, 1997. Evaluation of varietal susceptibility in peas to *Mycosphaerella pinodes*. Ann Appl Biol 130: 32–33.

Nasir, M., H.H. Hoppe & F. Ebrahim-Nesbat, 1992. The development of different pathotype groups of *Mycosphaerella pinodes* in susceptible and partially resistant pea leaves. Plant Pathol 41: 187–194.

Navas-Cortés, J.A., E. Pérez-Artés, R.M. Jiménez-Diaz, A. Llobell, B.W. Bainbridge & J.B. Heale, 1998. Mating type, pathotype and RAPDs analysis in *Didymella rabiei*, the agent of ascochyta blight of chickpea. Phytoparasitica 26: 199–212.

Nene, Y. & M.V. Reddy, 1987. Chickpea diseases and their control. In: M.C. Saxena & K.B. Singh (Eds.), The Chickpea, pp. 233–270. CAB International, Oxon, UK.

Nguyen, T.T., P.W.J. Taylor, J.B. Brouwer, E.C.K. Pang & R. Ford, 2001. A novel source of resistance in lentil (*Lens culinaris* ssp. *culinaris*) to ascochyta blight caused by *Ascochyta lentis*. Australasian Plant Pathol 30: 211–215.

Nirenberg, H.I., U. Feiler & G. Hagedorn, 2002. Description of *Colletotrichum lupini* Comb. Nov. in modern terms. Mycologia 94: 307–320.

O'Connell, A.B., Uronu, G. Waskman, C. Nash, J.P.R. Keon & J.A. Bailey, 1993. Hemibiotrophic infection of *Pisum sativum* by *Colletotrichum truncatum*. Plant Pathol 42: 774–783.

Ondrej, M., 1993. Response of resistant lines of horse bean to pathogenic fungus *Ascochyta fabae* Speg. Plant Genet Resources, Annu Rep 1992 2: 45–48.

Onfroy, C., B. Tivoli, R. Corbière & Z. Bouznad, 1999. Cultural, molecular and pathogenic variability of *Mycosphaerella pinodes* and *Phoma medicaginis* var. *pinodella* isolates from dried pea in France. Plant Pathol 48: 218–229.

Parlevliet, J.E., 1979. Components of resistance that reduce the rate of epidemic development. Annu Rev Phytopathol 17: 203–222.

Parlevliet, J.E., 1983. Can horizontal resistance be recognized in the presence of vertical resistance? In plants exposed to a mixture of pathogen races? Phytopathology 73: 379.

Parlevliet, J.E., 2002. Durability of resistance against fungal, bacterial and viral pathogens; present situation. Euphytica 124: 147–156.

Pedersen, E.A. & R.A.A. Morrall, 1994a. Effect of non-host and fungicide-treated barriers on horizontal spread of ascochyta blight on lentils. Can J Plant Pathol 16: 317–325.

Pedersen, E.A. & R.A.A. Morrall, 1994b. Effects of cultivar, leaf wetness duration, temperature and growth stage on infection and development of ascochyta blight of lentil. Phytopathology 84: 1024–1030.

Porta-Puglia, A., C.C. Bernier, G.J. Jellis, W.J. Kaiser & M.V. Reddy, 1994. Screening techniques and sources of resistance to foliar diseases caused by fungi and bacteria in cool season food legumes. Euphytica 73: 11–25.

Porta-Puglia, A., P. Crinò & C. Mosconi, 1996. Variability in virulence to chickpea of an Italian population of *Ascochyta rabiei*. Plant Dis 80: 39–41.

Prioul, S., C. Onfroy, B. Tivoli & A. Baranger, 2003. Controlled environment assessment of partial resistance to *Mycosphaerella pinodes* on pea (*Pisum sativum* L.) seedlings. Euphytica 131: 121–130.

Prioul, S., A. Frankewitz, G. Deniot, G. Morin & A. Baranger, 2004. Mapping of quantitative trait loci for partial resistance to *Mycosphaerella pinodes* on pea (*Pisum sativum* L.), at the seedling and adult plant stages. Theor Appl Genet 108: 1322–1334.

Pritchard, P.R., P.S. Rowe & S. Rossal, 1989. A comparison of infection of resistant and susceptible lines of field bean (*Vicia faba*) by *Ascochyta fabae*. Plant Pathol 38: 266–270.

Qureshi, S.H. & S.S. Alam, 1984. Pathogenic behaviour of *Ascochyta rabiei* isolates on different cultivars of chickpea in Pakistan. Int Chickpea Newslett 11: 29–31.

Ramsey, M., R. Knight & J. Paull, 1995. Asochyta and Chocolate Spot resistant faba beans (*Vicia faba* L.) for Australia. In: 2nd European Conference on Grain Legumes, 9–13 July, Copenhagen, Denmark, pp. 164–165.

Rapilly, F., 1991. L'épidémiologie en pathologie végétale: mycoses aériennes. Paris INRA, 317 pp.

Rashid, K.Y., C.C. Bernier & R.L. Conner, 1991a. Evaluation of faba bean for resistance to *Ascochyta fabae* and development of host differentials for race identification. Plant Dis 75: 852–855.

Rashid, K.Y., C.C. Bernier & R.L. Conner, 1991b. Genetics of resistance in faba bean inbred lines to five isolates of *Ascochyta fabae*. Can J Plant Pathol 13: 218–225.

Reddy, M.V. & S. Kabbabeh, 1985. Pathogenic variability in *Ascochyta rabiei* (Pass.) Lab. in Syria and Lebanon. Phytopathol Mediterr 24: 265–266.

Reddy, M.V. & K.B. Singh, 1984. Evaluation of a world collection of chickpea germplasm accessions for resistance to ascochyta blight. Plant Dis 68: 900–901.

Reddy, M.V. & K.B. Singh, 1992. Registration of five chickpea germplasm lines resistant to ascochyta blight. Crop Sci 32: 1079–1080.

Riahi, H., M.M. Harrabi, M.H. Halila & R.N. Strange, 1990. A quantitative scale for assessing chickpea reaction to *Ascochyta rabiei*. Can J Bot 68: 2736–2738.

Ribeiro do Vale, F.X., J.E. Parlevliet & L. Zambolim, 2001. Concepts in plant disease resistance. Fitopatol Brasil 26: 577–589.

Rubiales, D. & A. Trapero, 2002. Occurrence of *Didymella fabae*, the teleomorph of *Ascochyta fabae*, on faba bean straw in southern Spain. J Phytopathol 150: 146–148.

Russell, A.C., 1994. 'Rajah' lentil (*Lens culinaris* Medik). New Zealand J Crop Hort Sci 22: 469–470.

Sakar, D., F.J. Muehlbauer & J.M. Kraft, 1982. Techniques of screening peas for resistance to *Phoma medicaginis* var. *pinodella*. Crop Sci 22: 988–992.

Shankar, M., W.A. Cowling & M.W. Sweetingham, 1995. Variation and stability of virulence in isolates of *Diaporthe toxica*. In: 10th Biennial Australasian Plant Pathology Society Conference, Abstract No. 213, Lincoln University, Christchurch, New Zealand.

Shankar, M., W.A. Cowling & M.W. Sweetingham, 1996. The expression of resistance to latent infection by *Diaporthe toxica* in narrow-leafed lupin. Phytopathology 86: 692–697.

Shankar, M., A. Gregory, M.J. Kalkhoven, W.A. Cowling & M.W. Sweetingham, 1998. A competitive ELISA for detecting resistance to latent stem infection by *Diaporthe toxica* in narrow-leafed lupins. Aust Plant Pathol 27: 251–258.

Shankar, M., M.W. Sweetingham & W.A. Cowling, 2002. Identification of alleles at two loci controlling resistance to Phomopsis stem blight in narrow-leafed lupin (*Lupinus angustifolius* L.). Euphytica 125: 35–44

Sillero, J., C.M. Ávila, M.T. Moreno & D. Rubiales, 2001. Identification of resistance to *Ascochyta fabae* in *Vicia faba* germplasm. Plant Breed 120(6): 529–531.

Singh, K.B., 1987. Chickpea breeding. In: M.C. Saxena & K.B. Singh (Eds.), The Chickpea, pp. 127–162. CAB International, Wallingford, UK.

Singh, K.B., G.C. Hawtin, Y.L. Nene & M.V. Reddy, 1981. Resistance in chickpeas to *Ascochyta rabiei*. Plant Dis 65: 586–587.

Singh, G., K. Singh, A.S. Gill & J.S. Brar, 1982. Screening of lentil varieties/lines for blight resistance. Indian Phytopathol 35: 678–679.

Singh, I.S., J.P. Singh, A.K. Singh & M.P. Chauhan, 1994. Pant Lentil 4: A high yielding, rust, wilt and blight resistant variety for the North Western Plains of India. LENS Newslett 21: 8–9.

Strange, R.N., E. Gewiss, J. Gil, T. Millan, J. Rubio, K. Daly, M. Kharrat, M. Cherif, A. Rhaiem, S. Maden, S. Dolar & F. Dusunceli, 2004. Integrated control of blight of chickpea, *Cicer arietinum*, caused by the fungus *Ascochyta rabiei*: An overview. In: AEP (Ed.), 5th European Conference on Grain Legumes & 2nd International Conference on Legume Genomics and Genetics, pp. 71–76.

Sugha, S.K., B.M. Singh & S.K. Sharma, 1991. Performance of lentil varieties/germplasm lines against blight. LENS Newslett 18: 34–35.

Sundheim, L., 1973. *Botrytis fabae*, *Botrytis cinerea* and *Ascochyta fabae* on broad bean (*Vicia faba*) in Norway. Acta Agric Scand 23(1): 43–51.

Sutton, B.C., 1998. The Coelomycetes. CAB International, Wallingford, UK.

Sweetingham, M.W., 1990. Coping with brown spot and root rot of lupins. J Agric W Aust 31: 5–13.

Sweetingham, M.W., R.A.C. Jones & A.G.P. Brown, 1998. Diseases and Pests. In: J.S. Gladstones, C.A. Pettersen, C.A. Atkins & J. Hamblin (Eds.), Lupins as Crop Plants: Biology, Production and Utilization, pp. 263–290. CAB International, Wallingford, UK.

Talhinhas, P., S. Sreenivasaprasad, J. Neves-Martins & H. Oliveira, 2002. Genetic and morphological characterisation of *Colletotrichum acutatum* causing anthracnose of lupins. Phytopathology 92: 986–996.

Tar'an, B., L. Buchwaldt, A. Tullu, S. Banniza, T.D. Warkentin & A. Vandenberg, 2003a. Using molecular markers to pyramid genes for resistance to ascochyta blight and anthracnose in lentil (*Lens culinaris* Medik). Euphytica 134: 223–230.

Tar'an, B., T. Warkentin, D.J. Somers, D. Miranda, A. Vandenberg, S. Blade, S. Woods, D. Bing, A. Xue, D. Dekoeyer & G. Penner, 2003b. Quantitative trait loci for lodging resistance, plant height and partial resistance to mycosphaerella blight in field pea (*Pisum sativum* L.). Theor Appl Genet 107: 1482–1491.

Tekeoglu, M., D.K. Santra, W.J. Kaiser & F.J. Muehlbauer, 2000. Ascochyta blight resistance inheritance in three chickpea recombinant inbred line populations. Crop Sci 40: 1251–1256.

Thomas, G.J. & M.W. Sweetingham, 2004. Cultivar and environment influence the development of lupin anthracnose caused by *Colletotrichum lupini*. Aust Plant Pathol 33: 571–577.

Timmerman, G.M., T.J. Frew, A.C. Russell, T. Khan, R. Butler, M. Gilpin, S. Murray & K. Falloon, 2002. Mapping of partial resistance to field epidemics of ascochyta blight of pea. Crop Sci 42: 2100–2111.

Tivoli, B., 1994. Conséquences des attaques parasitaires sur l'élaboration du rendement des plantes à croissance indéterminée. In *Agrophysiologie du pois protéagineux*. INRA/ITCF/UNIP éditions, 280 pp, 199–219.

Tivoli, B., C. Béasse, E. Lemarchand & E. Masson, 1996. Effect of ascochyta blight (*Mycosphaerella pinodes*) on yield components of single pea (*Pisum sativum*) plants under field conditions. Ann Appl Biol 129: 207–216.

Tivoli, B., D. Berthelem, J. Le Guen & C. Onfroy, 1986. Comparison of some methods for evaluation of reaction of different winter faba bean genotypes to *Botrytis fabae*. FABIS Newslett 16: 46–51.

Tivoli, B., P. Berthelem, J. Le Guen & C. Onfroy, 1988. A study of the performance of certain faba bean genotypes in relation to *Botrytis fabae* and *Ascochyta fabae* in France. FABIS Newslett 21: 36–39.

Tivoli, B. & C. Onfroy, 1997. A methodology in growth chamber to screen the pea resistance to *Mycosphaerella pinodes* and *Phoma medicaginis* var. *pinodella*. In: Proceedings of the III International Food Legume Research Conference, 22–26 September 1997, Adelaide, Australia, p. 174.

Tivoli, B., B. Reynaud, N. Maurin, P. Berthelem & J. Le Guen, 1987. Comparison of some methods for evaluation of reaction of different faba bean genotypes to *Ascochyta fabae*. FABIS Newslett 17: 35–38.

Trapero-Casas, A. & W.J. Kaiser, 1992a. Development of *Didymella rabiei*, the teleomorph of *Ascochyta rabiei*, on chickpea straw. Phytopathology 82: 1261–1266.

Trapero-Casas, A. & W.J. Kaiser, 1992b. Influence of temperature, wetness period, plant age, and inoculum concentration on infection and development of ascochyta blight of chickpea. Phytopathology 82: 589–596.

Tripathi, H.S. & Y.P.S. Rathi, 2000. Resistance to Botrytis gray mould in chickpea: Screening technique and identification of resistance sources. J Mycol Plant Pathol 30: 231–232.

Tufail, M., M. Ahmad & A. Ali, 1995. Masoor-93: An ideal combination of characters for Punjab province, Pakistan. LENS Newslett 22: 50–52.

Tufail, M., I.A. Malik, M. Choudhary, M. Ashraf & M. Saleem, 1993. Genetic resources and Breeding of lentil in Pakistan. In: W. Erskine & M.C. Saxena (Eds.), Lentil in South Asia, pp. 58–75. ICARDA, Aleppo, Syria.

Tullu, A., L. Buchwaldt, M. Lulsdorf, S. Banniza, B. Barlow, A.E. Slinkard, A. Sarker, B. Tar'an, T. Warkentin & A. Vandenberg, 2005. Sources of resistance to anthracnose (*Colletotrichum truncatum*) in wild *Lens* species. Genet Res Crop Evol (in press).

Tullu, A., L. Buchwaldt, T. Warkentin, B. Tar'an & A. Vandenberg, 2003. Genetics of resistance to anthracnose and identification of AFLP and Rapid markers linked to the resistance gene in PI 320937 germplasm of lentil (*Lens culinaris* Medik.). Theor Appl Genet 106: 428–434.

Udupa, S.M. & M. Baum, 2003. Genetic dissection of pathotype-specific resistance to ascochyta blight disease in chickpea (*Cicer arietinum* L.) using microsatellite markers. Theor Appl Genet 106: 1196–1202.

Udupa, S.M., F. Weigand, M.C. Saxena & G. Kahl, 1998. Genotyping with RAPD and microsatellite markers resolves pathotype diversity in the ascochyta blight pathogen of chickpea. Theor Appl Genet 97: 299–307.

Vandenberg, A., F.A. Kiehn, C. Vera, R. Gaudiel, L. Buchwaldt, S. Dueck, R.A.A. Morrall, J. Wahab & A.E. Slinkard, 2002a. CDC Glamis lentil. Can J Plant Sci 82: 103–104.

Vandenberg, A., F.A. Kiehn, C. Vera, R. Gaudiel, L. Buchwaldt, S. Dueck, R.A.A. Morrall, J. Wahab & A.E. Slinkard, 2002b. CDC Grandora lentil. Can J Plant Sci 82: 105–106.

Vandenberg, A., F.A. Kiehn, C. Vera, R. Gaudiel, L. Buchwaldt, S. Dueck, R.A.A. Morrall, J. Wahab & A.E. Slinkard, 2002c. CDC Sovereign lentil. Can J Plant Sci 82: 107–108.

Vandenberg, A., F.A. Kiehn, C. Vera, R. Gaudiel, L. Buchwaldt, S. Dueck, R.A.A. Morrall, J. Wahab & A.E. Slinkard, 2002d. CDC Vantage lentil. Can J Plant Sci 82: 109–110.

Vandenberg, A., F.A. Kiehn, C. Vera, R. Gaudiel, L. Buchwaldt, S. Dueck, R.A.A. Morrall, J. Wahab & A.E. Slinkard, 2002e. CDC Robin lentil. Can J Plant Sci 82: 111–112.

Vandenberg, A., F.A. Kiehn, C. Vera, R. Gaudiel, L. Buchwaldt, K.J. Kirkland, R.A.A. Morrall, J. Wahab & A.E. Slinkard, 2001. CDC Milestone lentil. Can J Plant Sci 81: 113–114.

Vir, S. & J.S. Grewal, 1974. Physiologic specialization in *Ascochyta rabiei*, the causal organism of gram blight. Indian Phytopathol 27: 355–360.

Wang, H., S.F. Hwang, K.F. Chang, G.D. Turnbull & R.J. Howard, 2000. Characterization of *Ascochyta* isolates and susceptibility of pea cultivars to the ascochyta disease complex in Alberta. Plant Pathol 49: 540–545.

Weimer, J.L., 1952. Lupine anthracnose. United States Department of Agriculture Bulletin, No. 904, 17 pp.

Wells, H.D. & I. Forbes, 1967. Effects of temperature on growth of *Glomerella cingulata* in vitro and on its pathogenicity to *Lupinus angustifolius* genotypes. Phytopathology 57: 1309–1311.

Wiatr, K., I. Frencel, E. Lewartowska & P. Barzyk, 2003. New cultivars of yellow lupin with enhanced tolerance to anthracnose. Advances of agricultural sciences problem issues: Lupin high-protein plant of XXI century Issue 495. Polish Academy of Sciences, Warsaw.

Williamson, P.M., K. Sivasithamparan & W.A. Cowling, 1991. Formation of subcuticular coralloid hyphae by *Phomopsis leptostromiformis* upon latent infection of narrow leafed lupins. Plant Dis 75: 1023–1026.

Wroth, J.M., 1996. Host-pathogen relationships of the ascochyta blight (*Mycosphaerella pinodes* (Berk. & Blox) Vestergr.) disease of field pea (*Pisum sativum* L.). Ph.D. Thesis, University of Western Australia, Perth.

Wroth, J.M., 1998a. Possible role for wild genotypes of *Pisum* spp. to enhance ascochyta blight resistance in pea. Aust J Exp Agric 38: 469–479.

Wroth, J.M., 1998b. Variation in pathogenicity among and within *Mycosphaerella pinodes* populations collected from field pea in Australia. Can J Bot 76: 1955–1966.

Wroth, J.M., 1999. Evidence suggests that *Mycosphaerella pinodes* infection of *Pisum sativum* is inherited as a quantitative trait. Euphytica 107: 193–204.

Wroth, J.M. & T.N. Khan, 1999. Differential responses of field pea (*Pisum sativum* L.) to ascochyta blight (*Mycosphaerella pinodes*): Rating disease in the field. Aust J Agric Res 50: 601–615.

Xue, A.G. & T.D. Warkentin, 2001. Partial resistance to *Mycosphaerella pinodes* in field pea. Can J Plant Sci 81: 535–540.

Xue, A.G., T.D. Warkentin, B.D. Gossen, P.A. Burnett, A. Vandenberg & K.Y. Rashid, 1998. Pathogenic variation of western Canadian isolates of *Mycosphaerella pinodes* on selected *Pisum* genotypes. Can J Plant Pathol 20: 189–193.

Xue, A.G., T.D. Warkentin, M.T. Greeniaus & R.C. Zimmer, 1996. Genotypic variability in seedborne infection of field pea by *Mycosphaerella pinodes* and its relation to foliar disease severity. Can J Plant Pathol 18: 370–374.

Xue, A.G., T.D. Warkentin & E.O. Kenaschuk, 1997. Effects of timings of inoculation with *Mycosphaerella pinodes* and its relation to foliar disease severity. Can J Plant Pathol 18: 370–374.

Yang, H.A., J.G. Boersma, You, M., B.J. Buirchell & M.W. Sweetingham, 2004. Development and implementation of a sequence specific PCR marker linked to a gene conferring resistance to anthracnose disease in narrow-leafed lupin (*Lupinus angustifolius* L.). Mol Breed 14: 145–151.

Yang, H.A., M. Shankar, B.J. Buirchell, M.W. Sweetingham, C. Caminero & P.M.C. Smith, 2002. Development of molecular markers using MFLP linked to a gene conferring resistance to *Diaporthe toxica* in narrow-leafed lupin (*Lupinus angustifolius* L.). Theor Appl Genet 105: 265–270.

Yang, H.A. & M.W. Sweetingham, 1998. The taxonomy of *Colletotrichum* isolates associated with lupin anthracnose. Aust J Agric Res 49: 1213–1223.

Yang, H.A. & M.W. Sweetingham, 2002. Variation in morphology and pathogenicity of *Pleiochaeta setosa* isolates from *Lupinus* spp. and other legumes. Aust Plant Pathol 31(3): 273–280.

Yang, H.A., M.W. Sweetingham & W.A. Cowling, 1996. The leaf infection process and resistance to *Pleiochaeta setosa* in three lupin species. Aust J Agric Res 47: 787–799.

Ye, G., D.L. McNeil & G.D. Hill, 2000. Two major genes confer ascochyta blight resistance coming from *Lens orientalis*. New Zealand Plant Protect 53: 109–132.

Ye, G., D.L. McNeil & G.D. Hill, 2003. Inheritance of foliar resistance to ascochyta blight in lentil (*Lens culinaris*) New Zealand J Crop Hort Sci 31: 77–83.

Zimmer, R.C. & R.C. Sabourin, 1986. Determining resistance reactions of field pea cultivars at the seedling stage to *Mycosphaerella pinodes*. Phytopathology 76: 878–881.